EFSA CEF Panel (EFSA Panel on Fo od Contact Materials, Enzymes, Flavourings and Proce ssing Aids), 2015. Scientific Opinion on Flavouring Group Evalua tion 09, Revision 6 (FGE.09Rev6): Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols from chemical group 8 and 30, and an ester of a phenol derivative from chemical group 25

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 9, Revision 6 (FGE.09Rev6): Secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols from chemical group 8 and 30, and an ester of a phenol derivative from chemical group 25[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 22 flavouring substances in the Flavouring Group Evaluation 9, Revision 6, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances was considered to have genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure–activity relationships, intake from current uses, toxicological threshold of concern and available data on metabolism and toxicity. The present revision of FGE.09 includes the assessment of one additional flavouring substance, trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219]. The Panel concluded that the 22 substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.109, 07.202, 07.203, 07.219, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. However, based on mTAMDI calculations, for 11 flavouring substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949] more reliable exposure data are required for a re-evaluation. For two substances [FL-nos: 07.219 and 09.929] no use levels are available and these should be submitted. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have been considered. Specifications, including complete purity criteria and identity for the materials of commerce, have been provided for all candidate substances.

KEY WORDS

flavourings, alcohols, ketones, esters, secondary alicyclic, saturated, unsaturated, food safety, FGE.09

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[1] On request from the European Commission, Question No EFSA-Q-2015-00318, adopted on 9 September 2015.

[2] Panel members: Claudia Bolognesi, Laurence Castle, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, Sirpa Kärenlampi, Wim Mennes, Maria Rosaria Milana, André Penninks, Maria de Fátima Tavares Poças, Vittorio Silano, Andrew Smith, Christina Tlustos, Fidel Toldrá, Detlef Wölfle, Holger Zorn and Corina-Aurelia Zugravu. Correspondence: fip@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Flavourings, Ulla Beckman Sundh, Leon Brimer, Karl-Heinz Engel, Paul Fowler, Rainer Gürtler, Trine Husøy, Wim Mennes, Gerard Mulder and Harriet Wallin, for the preparatory work on this scientific opinion and hearing experts Vibe Beltoft and Karin Nørby and EFSA staff Annamaria Rossi, Maria Carfi and Maria Anastassiadou for the support provided to this scientific opinion.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 22 flavouring substances in the Flavouring Group Evaluation (FGE) 9, Revision 6 (FGE.09Rev6), using the Procedure referred to in Commission Regulation (EC) No 1565/2000 "(hereinafter 'the Procedure')". These flavouring substances belong to chemical groups 8, 25 and 30 of Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision of FGE.09, FGE.09Rev6, includes the assessment of one additional candidate substance, trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219], which was not included in FGE.09Rev5. This substance is an α,β-unsaturated carbonyl, for which concern for genotoxicity was ruled out in FGE.212Rev3.

FGE.09Rev6 deals with 22 candidate substances: secondary alicyclic saturated and unsaturated alcohols, ketones, one hemiketal ester and esters containing secondary alicyclic alcohols.

Two candidate substances [FL-nos: 07.203 and 07.255] possess one chiral centre and 15 substances [FL-nos: 02.075, 02.167, 06.136, 07.059, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] possess two or more chiral centres.

Fourteen candidate substances belong to structural class I, seven substances belong to structural class II and one to structural class III according to the decision tree approach presented by Cramer et al. (1978).

Fifteen flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the "Maximised Survey-derived Daily Intake" (MSDI) approach to estimate the per capita intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Flavour Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a "modified Theoretical Added Maximum Daily Intake" (mTAMDI) approach based on the normal use levels reported by the Flavour Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, intakes in Europe of the 14 flavouring substances belonging to structural class I range from 0.0012 to 830 µg per capita per day, intakes of the seven substances from structural class II range from 0.0085 to 530 µg per capita per day and intake of the substance from structural class III is 1.2 µg per capita per day. These intakes are all below the threshold of concern values for structural classes I, II and III of 1 800, 540 and 90 µg per person per day, respectively. For one substance [FL-no: 09.520] from structural class II the MSDI is 770 µg per capita per day, which is above the threshold of concern of 540 µg per person per day. For this substance a No

Observed Adverse Effect Level (NOAEL) is available, providing a sufficient margin of safety based on the MSDI approach.

The total combined intakes of candidate and supporting substances from structural classes I and II do not give rise to a safety concern.

For five of the candidate substances [FL-nos: 07.109, 07.202, 07.219, 07.255 and 09.870] it has been concluded that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for supporting substances. Genotoxicity data are available for only a limited number of the remaining flavouring substances in the present group and the genotoxicity cannot be assessed adequately. However, the data available do not preclude evaluation of the substances using the Procedure.

All candidate substances are expected to be metabolised to innocuous products at the estimated levels of use as flavouring substances.

It was noted that where toxicity data were available they were consistent with the conclusions in the present FGE using the Procedure.

It is considered that the 22 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances based on the default MSDI approach.

In order to determine whether the conclusion for the 22 candidate substances, which have been evaluated using the Procedure, can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications, including complete purity criteria and information on identity for the materials of commerce, have been provided for all flavouring substances.

Thus, for 22 flavouring substances evaluated using the Procedure, the Panel considered that the materials of commerce would not present a safety concern at their estimated levels of intake based on the MSDI approach [FL-nos: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.109, 07.202, 07.203, 07.219, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949].

The estimated intakes of 13 candidate substances in structural class I, based on the mTAMDI approach, ranged from 420 to 63 000 µg per person per day. For six substances [FL-nos: 09.154, 09.618, 09.619, 09.843, 09.935 and 09.949], the mTAMDI is above the threshold of concern of 1 800 µg per person per day. For seven substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 09.355, 09.621 and 09.870], the mTAMDI is below the threshold. The mTAMDIs of five substances assigned to structural class II [FL-nos: 07.059, 07.109, 07.202, 07.203 and 09.520] range from 1 600 to 8 700 µg per person per day, which are above the threshold of concern for structural class II substances of 540 µg per person per day. The mTAMDI estimates for one substance from structural class II [FL-no: 07.255] and for the one candidate substance in class III [FL-no: 06.136] are 320 and 0.075 µg per person per day, respectively, which are below the thresholds of their structural classes (540 and 90 µg per person per day). For all substances with mTAMDI values below their structural class thresholds, the Panel noted that they have been evaluated using the A-side of the Procedure.

For one flavouring substance [FL-no: 09.929] from structural class I and one flavouring substance [FL-no: 07.219] from structural class II, use levels are missing and an mTAMDI cannot be calculated for these two substances.

In conclusion, for 11 candidate substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949], for which the mTAMDIs are above the thresholds for their structural class, and for another two substances [FL-nos: 07.219 and 09.929], for which use levels are missing, further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavouring is regulated under Regulation (EC) No 1334/2008[4] of the European Parliament and Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of article 9(a) of this Regulation an evaluation and approval are required for flavouring substances.

The Union List of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012.[5] The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000.[6]

On 25 November 2010, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids adopted an opinion on Flavouring Group Evaluation 212, Revision 1 (FGE.212Rev1): α,β-unsaturated alicyclic ketones and precursors from chemical subgroup 2.6 of FGE.19.[7]

The Panel concluded that the argumentation of Industry to expand its conclusion for the six-carbon ring members of subgroup 2.6 also to the cyclopentenyl derivatives in this subgroup [FL-no: 07.033, 07.094, 07.112 and 07.140] was considered too limited, given the lack of support from experimental data. Therefore, additional genotoxicity tests are still required for the representative substance [FL-no: 07.112] already chosen by the Panel. Alternatively, a more thorough explanation (physico-chemical parameters; experimental underpinning) of the proposed similar reactivity of six- and five-membered ring substances should be provided by Industry.

The requested data have been submitted by the applicant.

In addition, the flavouring substance [FL-no: 07.219], trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one, was put in FGE.212 (former FGE.19, subgroup 2.6b: α,β-unsaturated aldehydes and ketones and precursors) because of its structure relationship with this group. Although the substance as such is not mentioned in the data submitted by the applicant, the submitted data are likely to be relevant for [FL-no: 07.219] as well.

Therefore, this request covers as well the re-evaluation of trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219].

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority (EFSA) to finalise its safety assessment of these flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.[6]

INTERPRETATION OF THE TERMS OF REFERENCE

As additional genotoxicity data have been submitted to support evaluation of trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219] with respect to genotoxic potential in FGE.212rev3. Subsequently, the European Commission requests EFSA to carry out a safety assessment in accordance with Commission Regulation (EC) No 1565/2000 for trans-3-methyl-2-(2-pentenyl)-2-

[4] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

[5] EC (European Commission), 2012. Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1–161.

[6] Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, p. 8–16.

[7] EFSA Journal 2011;9(3):1923.

cyclopenten-1-one [FL-no: 07.219].The Panel concluded that [FL-no: 07.219] does not give rise to concern with respect to genotoxicity and can accordingly now be evaluated through the Procedure in FGE.09Rev6.

ASSESSMENT

1. History of the evaluation of the substances in the present Flavouring Group Evaluation

The Flavouring Group Evaluation (FGE) 09, FGE.09, dealt with 10 candidate substances, nine secondary alicyclic saturated and unsaturated alcohols, ketones and esters containing secondary alicyclic alcohols, and an ester of a phenol carboxylic acid and a secondary alicyclic alcohol.

The first revision of FGE.09, FGE.09Rev1, included the assessment of five additional flavouring substances [FL-nos: 06.136, 09.154, 09.520, 09.929 and 09.935]. No new toxicity or metabolism data were provided for four of the five substances. For one substance [FL-no: 09.520] acute and short-term toxicity data and *in vitro* and *in vivo* genotoxicity data were provided. Additional data on five substances [FL-nos: 02.075, 02.167, 09.355, 09.619 and 09.621] were made available since FGE.09 was published.

The second revision of FGE.09, FGE.09Rev2, included the assessment of one additional substance, carvyl-3-methylbutyrate [FL-no: 09.870]. No toxicity and/or metabolism data were provided for this substance. Carvyl-3-methylbutyrate has initially been considered in FGE.212 with respect to genotoxicity, together with other α,β-unsaturated substances from subgroup 2.6 of FGE.19, where the Panel concluded that "*d*-Carvone [FL-no: 07.146] was found genotoxic *in vitro*. However, *d*-carvone was not carcinogenic in mice. Therefore, the Panel concluded that this substance together with the structurally related *l*-carvone as well as carveol and the carvyl derivatives [FL-nos: 02.062, 07.147, 09.143, 09.215 and 09.870] could be evaluated through the Procedure".

The third revision of FGE.09, FGE.09Rev3, included the assessment of one additional substance, L-menthyl (*S*)-3-hydroxybutyrate [FL-no: 09.949]. No toxicity and/or metabolism data were provided for this substance.

The fourth revision of FGE.09, FGE.09Rev4, included the assessment of four additional substances [FL-nos: 07.059, 07.202, 07.255 and 09.843]. Two of these substances [FL-nos: 07.202 and 07.255] are α,β-unsaturated ketones originally allocated to FGE.212Rev1. The two substances were considered with respect to genotoxicity (EFSA CEF Panel, 2011) and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly the two substances could be evaluated through the Procedure. No toxicity or metabolism data were provided for the new substances. Since the publication of FGE.09Rev3, one substance (former candidate substance [FL-no: 07.207]) is no longer supported for use as a flavouring substance in Europe by the flavour industry and will therefore not be considered any further (EFFA, 2009). Information from the previous version of FGE.09 on this substance is to be kept in the main text only if relevant for the remaining candidate substances.

The fifth revision of FGE.09, FGE.09Rev5, included the assessment of one additional substance, 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109]. This substance is an α,β-unsaturated ketone originally allocated to FGE.213. The substance was considered with respect to genotoxicity in FGE.213Rev1 (EFSA CEF Panel, 2014) and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly the substance could be evaluated through the Procedure. No toxicity or metabolism data were provided for the substance.

FGE	Opinion adopted by EFSA	Link	No of candidate substances
FGE.09	9 December 2004	http://www.efsa.eu.int/science/afc/afc_opinions/814_en.html	10
FGE.09Rev1	1 April 2008	http://www.efsa.europa.eu/en/efsajournal/doc/927.pdf	15
FGE.09Rev2	13 May 2009	http://www.efsa.europa.eu/en/efsajournal/pub/1454.htm	16
FGE.09Rev3	28 September 2011	http://www.efsa.europa.eu/en/efsajournal/pub/2396.htm	17
FGE.09Rev4	9 July 2012	http://www.efsa.europa.eu/en/efsajournal/pub/2836.htm	21

FGE	Opinion adopted by EFSA	Link	No of candidate substances
FGE.09Rev5	25 September 2014	http://www.efsa.europa.eu/en/efsajournal/doc/3865.pdf	21
FGE.09Rev6	9 September 2015	http://www.efsa.europa.eu/en/efsajournal/doc/4243.pdf	22

The present revision of FGE.09, FGE.09Rev6, includes the assessment of one additional substance [FL-no: 07.219]. This substance is an α,β-unsaturated ketone originally allocated to FGE.212. The substance has been considered with respect to genotoxicity in FGE.212Rev3 (EFSA CEF Panel, 2015) and the Panel concluded that the data available ruled out the concern for genotoxicity and accordingly the substance can be evaluated through the Procedure. No toxicity or metabolism data were provided for this candidate substance. A search in the open literature for the substance did not provide any further data on toxicity or metabolism.

2. Presentation of the substances in Flavouring Group Evaluation 09, Revision 6

2.1. Description

The present FGE (FGE.09Rev6), using the Procedure referred to in Commission Regulation (EC) No 1565/2000 (for a schematic form see Appendix A), deals with 10 secondary alicyclic saturated and unsaturated alcohols, ketones, one hemiketal ester and 11 esters containing secondary alicyclic alcohols. These 22 flavouring substances (candidate substances) belong to chemical groups 8, 25 and 30 of Annex I of Regulation (EC) No 1565/2000.

The candidate substances under consideration, with their chemical register names, FLAVIS (FL), Chemical Abstract Service (CAS), Council of Europe (CoE) and Flavor and Extract Manufacturers Association (FEMA) numbers, structure and specifications, are listed in Table 1.

A summary of the outcome of the safety evaluation of the candidate substances is listed in Table 6.

The hydrolysis products of the candidate substances and their evaluation status as flavouring substances are listed in Table 7.

The candidate substances are structurally related to 27 flavouring substances (supporting substances) evaluated at the 51st, 59th and 63rd meetings of the Joint (Food and Agriculture Organization of the United Nations (FAO)/(World Health Organization (WHO) Expert Committee on Food Additives (JECFA, 2000a, 2003, 2005a, b) in the groups “Substances structurally related to menthol”, “Carvone and structurally related substances”, “Alicyclic ketones, secondary alcohols and related esters” and “Monocyclic and bicyclic secondary alcohols, ketones and related esters” (JECFA, 1999a, 2003, 2006). In addition the racemate of menthyl-3-hydroxybutyrate has been evaluated by the JECFA at the 69th meeting (JECFA, 2009) in the group of “Substances structurally related to menthol”.

The names and structures for the 27 supporting substances are listed in Table 8, together with their evaluation status.

SUMMARY OF SPECIFICATION DATA

Table 1: Specification Summary of the Substances in the FGE.09Rev6

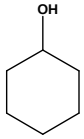
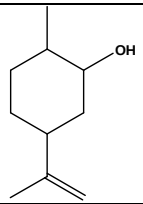
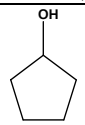
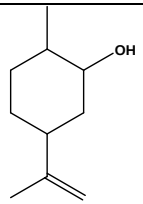
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	Specification comments
02.070	Cyclohexanol		2138 108-93-0	Solid $C_6H_{12}O$ 100.16	Slightly soluble Freely soluble	158 25 MS 95 %	1.462–1.468 0.942–0.948	
02.075	(1*R*,2*S*,5*S*)-neo-Dihydrocarveol		2296 18675-33-7	Liquid $C_{10}H_{18}O$ 154.25	Practically insoluble or insoluble Freely soluble	107 (33 hPa) MS 95 %	1.476–1.482 0.920–0.926	
02.135	Cyclopentanol		10193 96-41-3	Liquid $C_5H_{10}O$ 83.13	Slightly soluble Freely soluble	140 MS 95 %	1.449–1.455 0.945–0.951	
02.167	(1*R*,2*R*,5*S*)-Isodihydrocarveol		18675-35-9	Liquid $C_{10}H_{18}O$ 154.25	Practically insoluble or insoluble Freely soluble	90 (6.7 hPa) MS 95 %	1.475–1.481 0.918–0.924	

Table 1: Specification Summary of the Substances in the FGE.09Rev6

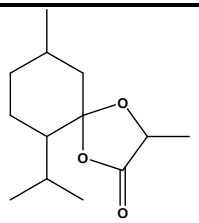
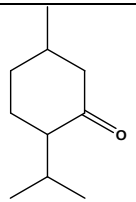
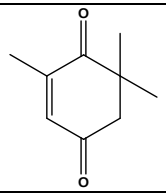
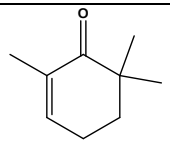
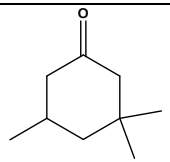
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	Specification comments
06.136 1859	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one		4285 831213-72-0	Liquid $C_{13}H_{22}O_3$ 226.32	Slightly soluble Soluble	259 IR NMR MS 98.9 %	1.4606–1.4609 1.017–1.021	Mixture of isomers: (3*S*, 5*R*, 6*S*,9*R*)-isomer: 65.6 % and (3*S*, 5*S*, 6*S*,9*R*)-isomer: 27.4 %, mixture of other diastereomers: 5.86 % (Flavour Industry, 2006b)
07.059	*p*-Menthan-3-one		2667 2035 10458-14-7	Liquid $C_{10}H_{18}O$ 154.25	Soluble Soluble	207 MS 96 %	1.448–1.453 0.888–0.895	Mixture of diastereomers, approximately 25 % of each (EFFA, 2012)
07.109 1857	2,6,6-Trimethylcyclohex-2-en-1,4-dione		3421 11200 1125-21-9	Solid $C_9H_{12}O_2$ 152.2	Slightly soluble Soluble	222 23–28 IR NMR 98 %	n.a. n.a.	
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		20013-73-4	Liquid $C_9H_{14}O$ 138.21	Slightly soluble Freely soluble	63 (16 hPa) MS 95 %	1.470–1.476 0.924–0.930	
07.203	3,3,5-Trimethylcyclohexan-1-one		873-94-9	Liquid $C_9H_{16}O$ 140.22	Practically insoluble or insoluble Freely soluble	189 MS 95 %	1.442–1.448 0.888–0.894	Racemate (EFFA, 2010a, b)

Table 1: Specification Summary of the Substances in the FGE.09Rev6

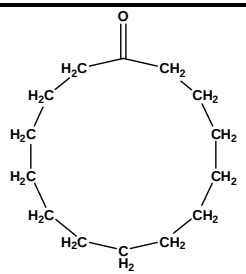
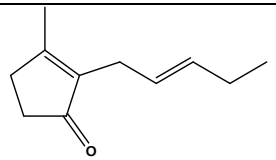
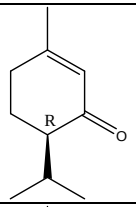
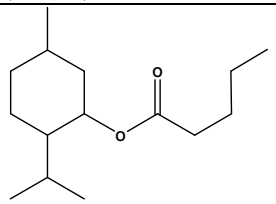
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	Specification comments
07.207	Cyclotetradecanone		3603-99-4	Solid $C_{14}H_{26}O$ 210.36	Practically insoluble or insoluble Freely soluble	159 (16 hPa) 53 NMR 95 %	n.a. n.a.	No longer supported by the Flavour Industry (EFFA, 2009)
07.219	trans-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one		3196 11786 6261-18-3	Liquid $C_{11}H_{16}O$ 164.25	Soluble Soluble	248 MS 98 %	1.495–1.501 0.942–0.948	
07.255 1856	l-Piperitone		4200 4573-50-6	Liquid $C_{10}H_{16}O$ 152.24	Slightly soluble Freely soluble	246 MS 99 %	1.482–1.488 0.929–0.935	
09.154 1852	Menthyl valerate		4156 472 89-47-4	Liquid $C_{15}H_{28}O_2$ 240.39	Practically insoluble or insoluble Freely soluble	261 NMR 95 %	1.445–1.451 0.903–0.909	Register name to be changed to (1*R*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexyl valerate (EFFA, 2010a)

Table 1: Specification Summary of the Substances in the FGE.09Rev6

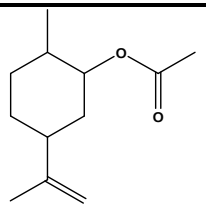
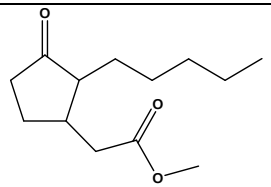
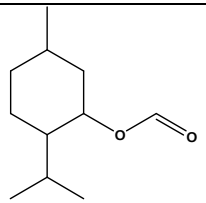
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	Specification comments
09.355	neo-Dihydrocarvyl acetate		10859 56422-50-5	Liquid $C_{12}H_{20}O_2$ 196.29	Practically insoluble or insoluble Freely soluble	266 MS 95 %	1.453–1.459 0.925–0.931	According to EFFA: Mixture of the two racemic forms (1*S*,2*R*,5*R*) and (1*R*,2*S*,5*S*), which is specified by the name (EFFA, 2005)
09.520 1898	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate		3408 10785 24851-98-7	Liquid $C_{13}H_{22}O_3$ 226.32	Slightly soluble Freely soluble	111 (0.1 hPa) NMR MS 98 %	1.458–1.462 0.997–1.006	According to EFFA: Mixture of the four stereoisomeric forms (*RR*, *RS*, *SR* and *SS*) in relatively equal ratios (approximately 25 % of each) (EFFA, 2010a)
09.618	Menthyl formate		10751 2230-90-2	Liquid $C_{11}H_{20}O_2$ 184.28	Practically insoluble or insoluble Freely soluble	95 (13 hPa) 9 MS 95 %	1.446–1.452 0.933–0.939	According to EFFA: Mixture of the two racemic forms (1*S*,2*R*,5*S*) and (1*R*,2*S*,5*R*), which is specified by the name (EFFA, 2010b)

Table 1: Specification Summary of the Substances in the FGE.09Rev6

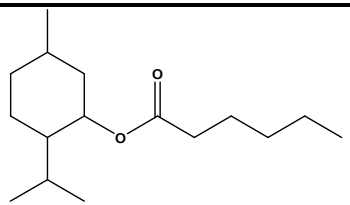
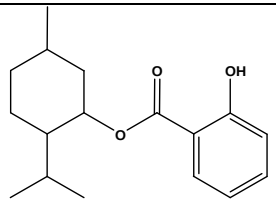
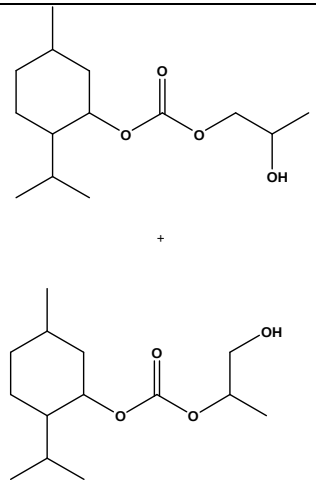
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility [(a)] Solubility in ethanol [(b)]	Boiling point, °C [(c)] Melting point, °C ID test Assay minimum	Refrac. Index [(d)] Spec.gravity [(e)]	Specification comments
09.619	(1*R*,2*S*,5*R*)-Menthyl hexanoate		6070-16-2	Liquid $C_{16}H_{30}O_2$ 254.14	Practically insoluble or insoluble Freely soluble	153 (20 hPa) MS 95 %	1.445–1.451 0.898–0.906	
09.621	(1*R*,2*S*,5*R*)-Menthyl salicylate		89-46-3	Liquid $C_{17}H_{24}O_3$ 276.37	Practically insoluble or insoluble Freely soluble	175 (13 hPa) MS 95 %	1.509–1.515 1.047–1.053	
09.843	Menthol 1- and 2-propylene glycol carbonate		3806 30304-82-6	Liquid $C_{14}H_{26}O_4$ 258.36	Insoluble Soluble	143 IR MS 98 %	1.458–1.458 1.013–1.014	According to EFFA: [FL-no: 09.843] is a mixture of 60 % menthol 1-propylene glycol carbonate (which is a mixture of four stereoisomers, 15 % of each) and 40 % menthol 2-propylene glycol carbonate (which is a mixture of four stereoisomers 10 % of each) (EFFA, 2012)

Table 1: Specification Summary of the Substances in the FGE.09Rev6

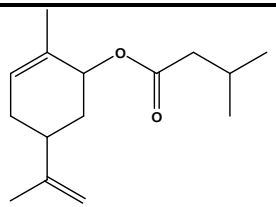
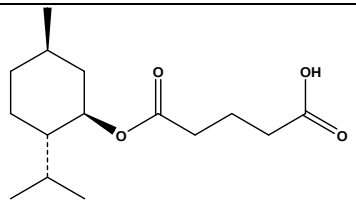
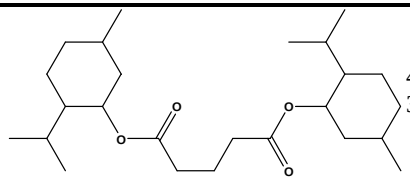
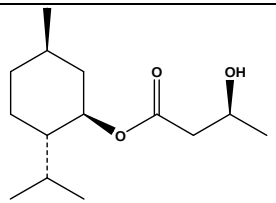
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	Specification comments
09.870	Carvyl-3-methylbutyrate		94386-39-7	Liquid $C_{15}H_{24}O_2$ 236.37	Practically insoluble or insoluble Freely soluble	343 MS 95 %	1.462–1.468 0.932–0.938	According to EFFA: mixture of the four stereoisomeric forms (*RR*, *RS*, *SR* and *SS*) in relatively equal ratios (approximately 25 % of each) (EFFA, 2010a)
09.929	L-Monomenthyl glutarate		4006 220621-22-7	Liquid $C_{15}H_{26}O_4$ 270	Sparingly soluble Soluble	390 (decomp) n.a. IR NMR 95 %	1.462–1.470 1.026–1.036	

Table 1: Specification Summary of the Substances in the FGE.09Rev6

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility [a] Solubility in ethanol [b]	Boiling point, °C [c] Melting point, °C ID test Assay minimum	Refrac. Index [d] Spec.gravity [e]	Specification comments
09.935	Dimenthyl glutarate		406179-71-3	Solid $C_{25}H_{44}O_4$ 408	Insoluble Soluble	48–50 NMR MS 98 %	n.a. n.a.	According to EFFA: Menthyl moiety mixture of the two racemic forms (1*S*,2*R*,5*S*) and (1*R*,2*S*,5*R*), which is specified by the name. Since there are two menthyl moieties, three combinations exist, approximately 25 % (+/+), 25 % (–/–) and 50 % (+/–) (EFFA, 2010a)
09.949	L-Menthyl (*S*)-3-hydroxybutyrate		4308 115869-76-6	Liquid $C_{14}H_{26}O_3$ 242.35	Slightly soluble Soluble	95–97 (0.7 hPa) IR MS 98 %	1.454–1.464 0.969–0.979	Stereoisomeric composition of (*S*)-form: > 80 % ee and (*R*)-form < 20 % ee (ee = enantiomeric excess)

ID, identity; IR, infrared spectroscopy; MS, mass spectrometry; n.a., not applicable; NMR, nuclear magnetic resonance.
(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95 % ethanol, if not otherwise stated.
(c): At 1 013.25 hPa, if not otherwise stated.
(d): At 20 °C, if not otherwise stated.
(e): At 25 °C, if not otherwise stated.

2.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, and they may have different chemical properties, resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.).

Two candidate substances possess one chiral centre [FL-nos: 07.203 and 07.255] and 15 substances possess two or more chiral centres [FL-nos: 02.075, 02.167, 06.136, 07.059, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] (Table 1).

2.3. Natural occurrence in food

Fifteen candidate substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 07.059, 07.109, 07.202, 07.203, 07.219, 07.255, 09.154, 09.355, 09.520, 09.618 and 09.619] have been reported to occur in fruits, spices, butter, chicken, wine, drinks, tea, juice and essential oils. Quantitative data on the natural occurrence of these substances in food have been reported for four substances (TNO, 2000, 2011, 2014, 2015) (Table 2).

Table 2: Candidate substances for which quantitative information on occurrence in food is available

FL-no	Name	Quantitative data reported
02.070	Cyclohexanol	Up to 0.1 mg/kg in fruits (passionfruit) and 0.006 mg/kg in white wine
02.135	Cyclopentanol	0.01–0.1 mg/kg in passiflora juice, 0.01–0.1 mg/kg in *Passiflora mollissima*, 0.01–0.02 mg/kg in oysters and 0.01 mg/kg in Chinese quince flesh
07.109	2,6,6-Trimethylcyclohex-2-en-1,4-dione	Up to 8 mg/kg in honey for consumption and up to 9 mg/kg in tea
07.202	2,6,6-Trimethylcyclohex-2-en-1-one	2 000 mg/kg in maize, 1 mg/kg in tea and up to 0.23 mg/kg in citrus fruits

According to Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek (TNO) the remaining seven candidate substances have not been reported to occur naturally in any food items (TNO, 2000, 2010, 2011) (Table 3).

Table 3: Candidate substances not reported to occur naturally in food

FL-no	Name
06.136	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one
09.621	Menthyl salicylate
09.843	Menthol 1-and 2-propylene glycol carbonate
09.870	Carvyl-3-methylbutyrate
09.929	L-Monomenthyl glutarate
09.935	Dimenthyl glutarate
09.949	L-Menthyl (*S*)-3-hydroxybutyrate

3. Specifications

Purity criteria for the 22 candidate substances have been provided by the Flavour Industry (EFFA, 2003a, 2010a, 2011; Flavour Industry, 2004, 2006a, b, 2007, 2010a).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000, the specifications are adequate for all substances (see Section 2.2 and Table 1).

4. Intake data

Annual production volumes of the flavouring substances as surveyed by the Flavour Industry can be used to calculate the "Maximised Survey-derived Daily Intake" (MSDI) by assuming that the production figure represents only 60 % of the use in food because of underreporting and that 10 % of the total European Union (EU) population are consumers (SCF, 1999).

However, the Panel noted that because of year-to-year variability in production volumes, uncertainties in the underreporting correction factor and uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that, in contrast to the generally low per capita intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds, below which exposures are not considered to present a safety concern, might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the Scientific Committee on Food (SCF) recommended also taking into account the results of other intake assessments (SCF, 1999).

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g. it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004).

4.1. Estimated daily per capita intake (MSDI Approach)

The intake estimation is based on the MSDI approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavor Industry (IOFI), in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average per capita intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[8] (Eurostat, 1998). This is derived for candidate substances from

[8] EU figure: 375 million. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

estimates of annual volume of production provided by the Flavour Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Flavour Industry surveys (SCF, 1999).

In the present FGE.09Rev6, the total annual volume of production of the candidate substances from use as flavouring substances in Europe has been reported to be approximately 19 600 kg (EFFA, 2003a, b, 2011; Flavour Industry, 2004, 2006a, b, 2007, 2010a, b). For 26 of the 27 supporting substances, for which production figures are available for Europe, the total annual volume of production is approximately 138 500 kg (JECFA, 1999a, 2003, 2006).

On the basis of the annual volumes of production reported for the candidate substances, the MSDI values for each of these flavourings have been estimated (Table 6).

Ninety-six per cent of the total annual volumes of production for the candidate substances is accounted for by four of these flavourings, *p*-menthan-3-one [FL-no: 07.059], methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520], menthol 1- and 2-propylene glycol carbonate [FL-no: 09.843] and *L*-monomenthyl glutarate [FL-no: 09.929]. The estimated daily intakes from use as flavouring substances are 530, 770, 830 and 110 µg per capita per day, respectively. The intakes for each of the remaining substances are 50 µg per capita per day or below (Table 6).

4.2. Intake estimated on the basis of the modified TAMDI (mTAMDI)

The method for calculation of the modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For the present evaluation of the candidate substances, information on food categories and normal and maximum use levels[9,10,11] were submitted by the Flavour Industry (Burdock, 1995; EFFA, 2003a, 2007; Flavour Industry, 2004, 2006a, b, 2007, 2010a, b). For 20 candidate substances, the use in flavoured food products divided into the food categories as outlined in Annex III of the Commission Regulation (EC) No 1565/2000, is shown in Table 4. For trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219] and *L*-monomenthyl glutarate [FL-no: 09.929] the use levels have not been reported in accordance with the Commission Regulation.

For the present calculation of the mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 4: Use of 20 candidate substances for which data on use have been provided

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All except one [FL-no: 07.059]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except four [FL-nos: 07.059, 07.255, 09.843 and 09.949]
03.0	Edible ices, including sherbet and sorbet	All

[9] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002).
[10] The normal and maximum use levels in different food categories [Reg. (EC) No 1565/ 2000] have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004).
[11] The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007).

Table 4: Use of 20 candidate substances for which data on use have been provided

Food category	Description	Flavourings used
04.1	Processed fruits	All except four [FL-nos: 06.136, 07.059, 07.255 and 09.843]
04.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds	Only one [FL-no: 09.935]
05.0	Confectionery	All
06.0	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery	All except four [FL-nos: 07.059, 07.109, 07.255 and 09.935]
07.0	Bakery wares	All
08.0	Meat and meat products, including poultry and game	All except five [FL-nos: 06.136, 07.059, 07.255, 09.843 and 09.949]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except five [FL-nos: 06.136, 07.059, 07.255, 09.843 and 09.949]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products, etc.	All except two [FL-nos: 06.136 and 07.059]
13.0	Foodstuffs intended for particular nutritional uses	All except five [FL-nos: 07.059, 07.255, 09.843, 09.935 and 09.949]
14.1	Non-alcoholic (“soft”) beverages, excluding dairy products	All except one [FL-no: 09.870]
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts	All
15.0	Ready-to-eat savouries	All except two [FL-nos: 06.136 and 07.059]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat)—foods that could not be placed in categories 1–15	All except two [FL-nos: 07.059 and 09.843]

For the candidate substances for which the Flavour Industry has provided data on food categories, normal use levels are in the range of 0.0001–500 mg/kg food, and the maximum use levels are in the range of 0.0001–2000 mg/kg food (Burdock, 1995; EFFA, 2003a, 2007; Flavour Industry, 2004, 2006a, b, 2007, 2010a, b).

The mTAMDI values for 13 candidate substances from structural class I (see Section 7) for which exposure data have been submitted range from 420 to 63 000 µg per person per day. For the six candidate substances from structural class II, the mTAMDI values range from 320 to 8 700 µg per person per day. For the remaining substance from structural class III the mTAMDI is 0.075 µg per person per day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 7 and Appendix C.

5. Absorption, distribution, metabolism and elimination

The 11 esters [FL-nos: 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] included in this FGE are expected to be hydrolysed to the corresponding carboxylic acids and alcohols, based on the data available for the supporting substances (White et al., 1990; Emberger, 1994a, b). The resulting carboxylic acids are either metabolised through common physiological pathways, such as beta-oxidation and the citric acid cycle, or excreted in conjugation with glucuronide (Keefer et al., 1987; Vree et al., 1994) (see Table 7 and Appendix D).

The one hemiketal ester [FL-no: 06.136] is expected to be hydrolysed to the corresponding cyclic ketone, *p*-menthan-3-one [FL-no: 07.059], and lactic acid [FL-no: 08.004].

One of the main pathways for the candidate alcohols and the ketones (after reduction) [FL-nos: 02.070, 02.075, 02.135, 02.167, 07.059, 07.109, 07.202, 07.203, 07.219 and 07.255] is conjugation with glucuronic acid followed by excretion. Menthol, carveol and dihydrocarveol, the hydrolysis products of 11 substances [FL-nos: 06.136, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] are also metabolised via this pathway. Menthol, carveol and dihydrocarveol are not anticipated to be oxidised to the corresponding ketone (for detailed discussion, see Appendix D).

Additional pathways involved in the metabolism of the candidate substances are reduction of ketone groups, oxidation of alkyl groups of alkyl substituted alicyclic ketones followed by conjugation with glucuronic acid and/or sulphates resulting in excretion (see Appendix D). Thus, it may be anticipated that these 22 substances will be metabolised to innocuous products.

A more detailed description of the metabolism is given in Appendix D.

6. Application of the procedure for the safety evaluation of flavouring substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment, using the mTAMDI approach, is not carried out using the Procedure. In these cases, the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 7.

For the safety evaluation of the candidate substances the Procedure was applied. The stepwise evaluations are summarised in Tables 5 and 6.

Step 1

Fourteen of the candidate substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] are classified into structural class I, seven candidate substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 07.219, 07.255 and 09.520] into structural class II and one substance [FL-no: 06.136] is classified into structural class III according to the decision tree approach presented by Cramer et al. (1978).

Step 2

Step 2 requires consideration of whether detoxification pathways are available to metabolise the substances, at the estimated levels of intake, to innocuous products.

All the candidate substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.109, 07.202, 07.203, 07.219, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] are expected to be metabolised to innocuous products and, accordingly, they proceed via the A-side of the Procedure scheme (Section 5 and Appendix D).

Step A3

For the 14 candidate substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] that have been assigned to structural class I, estimated European daily per capita intakes range from 0.0012 to 830 µg (Table 6). These intakes are below the threshold of concern of 1 800 µg per person per day for structural class I.

For the seven candidate substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 07.219, 07.255 and 09.520] assigned to structural class II, European daily per capita intakes range from 0.0085 to 770 µg. For six of these [FL-nos: 07.059, 07.109, 07.202, 07.203, 07.219 and 07.255] the intakes are below the threshold of concern of 540 µg per person per day for structural class II. For one substance [FL-no: 09.520] the daily per capita intake of 770 µg is above the threshold of concern for a substance assigned to structural class II. The substance therefore proceeds to step A4.

One candidate substance [FL-no: 06.136] has been assigned to structural class III and its European daily per capita intake is 1.2 µg. This intake is below the threshold of concern of 90 µg per person per day for structural class III.

Based on the results of the safety evaluation sequence, 21 candidate substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.109, 07.202, 07.203, 07.219, 07.255, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] proceeding via the A-side of the Procedure do not pose a safety concern when used at estimated levels of intake, based on the MSDI approach, as flavouring substances.

Step A4

Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] and its metabolites are not endogenous; therefore, this substance proceeds to step A5.

Step A5

A 90-day study in rats has been performed for one substance [FL-no: 09.520] from which a No Observed Adverse Effect Level (NOAEL) of 100 mg/kg body weight (bw) per day could be derived. This NOAEL provides a margin of safety of 7 700 compared with the daily intake of 0.013 mg/kg bw per day for methyl 3-oxo-2-pentyl-1-cyclopentylacetate. Therefore, this substance [FL-no: 09.520] does not pose a safety concern when used at estimated levels of intake, based on the MSDI approach, as flavouring substance.

7. Comparison of the intake estimations based on the MSDI and the mTAMDI approach

The estimated intakes, based on the mTAMDI, range from 420 to 63 000 µg per person per day for 13 candidate substances in structural class I. For seven of these substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 09.355, 09.621 and 09.870] the mTAMDI is below the threshold of concern of 1 800 µg per person per day and for six substances [FL-nos: 09.154, 09.618, 09.619, 09.843, 09.935 and 09.949] the mTAMDI is above the threshold. For L-monomenthyl glutarate [FL-no: 09.929] no mTAMDI could be calculated due to lack of information on use levels in the food categories as outlined in Annex III of Commission Regulation (EC) No 1565/2000.

For six substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 07.255 and 09.520] assigned to structural class II, the estimated intakes, based on the mTAMDI, range from 320 to 8 700 µg per person per day, which are all above the threshold of concern for structural class II substances of 540 µg per person per day, except for one substance [FL-no: 07.255]. For the seventh substance assigned to class II, trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219], no mTAMDI could be calculated due to lack of information on use levels in the food categories as outlined in Annex III of Commission Regulation (EC) No 1565/2000.

For one substance [FL-no: 06.136], assigned to structural class III, the estimated intake based on the mTAMDI is 0.075 µg per person per day, which is below the threshold of concern for a structural class II substance of 90 µg per person per day.

Thus, for 10 candidate substances [FL-nos: 07.059, 07.202, 07.203, 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949], further information is required. This would include more reliable intake data and then, if required, additional toxicological data. For trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219] and L-monomenthyl glutarate [FL-no: 09.929] use levels are needed in accordance with the food categories as outlined in Annex III of Commission Regulation (EC) No 1565/2000.

For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, see Table 5.

Table 5: Estimated Intakes based on the MSDI Approach and the mTAMDI Approach

FL-no	EU Register name	MSDI (µg per capita per day)	mTAMDI (µg per person per day)	Structural class	Threshold of concern (µg per person per day)
02.070	Cyclohexanol	3.7	1 600	Class I	1 800
02.075	(1*R*,2*S*,5*S*)-neo-Dihydrocarveol	2.4	1 600	Class I	1 800
02.135	Cyclopentanol	0.012	1 600	Class I	1 800
02.167	(1*R*,2*R*,5*S*)-Isodihydrocarveol	2.4	1 600	Class I	1 800
09.154	Menthyl valerate	1	3 900	Class I	1 800
09.355	neo-Dihydrocarvyl acetate	0.012	1 600	Class I	1 800
09.618	Menthyl formate	0.73	3 900	Class I	1 800
09.619	(1*R*,2*S*,5*R*)-Menthyl hexanoate	0.37	3 900	Class I	1 800
09.621	(1*R*,2*S*,5*R*)-Menthyl salicylate	0.012	420	Class I	1 800
09.843	Menthol 1-and 2-propylene glycol carbonate	830	63 000	Class I	1 800
09.870	Carvyl-3-methylbutyrate	0.0012	1 000	Class I	1 800
09.929	L-Monomenthyl glutarate	110		Class I	1 800
09.935	Dimenthyl glutarate	30	38 000	Class I	1 800
09.949	L-Menthyl (*S*)-3-hydroxybutyrate	37	10 600	Class I	1 800
07.059	*p*-Menthan-3-one	530	8 700	Class II	540
07.109	2,6,6-Trimethylcyclohex-2-en-1,4-dione	50	1 900	Class II	540
07.202	2,6,6-Trimethylcyclohex-2-en-1-one	0.12	1 600	Class II	540
07.203	3,3,5-Trimethylcyclohexan-1-one	0.0085	1 600	Class II	540
07.219	trans-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	4.7		Class II	540
07.255	l-Piperitone	12	320	Class II	540
09.520	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate	770	3 900	Class II	540
06.136	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one	1.2	0.075	Class III	90

8. Considerations of combined intakes from use as flavouring substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be

considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. At present, the combined intake estimates are based only on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily per capita intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2003a, b, 2011; Flavour Industry, 2004, 2006a, b, 2007, 2010a, b), the combined estimated daily per capita intake as flavourings of the 14 candidate substances assigned to structural class I is 1 000 µg, which does not exceed the threshold of concern of 1 800 µg per person per day.

The candidate substances from structural class I are structurally related to 15 supporting substances for which European intake data are available (European intake data are available for only 15 of the 16 supporting substances from structural class I). The total combined intake of the 14 candidate and 15 supporting substances is approximately 17 000 µg per capita per day, which is above the threshold for structural class I substances of 1 800 µg per person per day. The major contribution (92 %) is provided by one supporting substance, menthol [FL-no: 02.015] (16 mg per capita per day), for which an Acceptable Daily Intake (ADI) of 0–4 mg/kg bw was allocated by the JECFA at its 51st meeting (JECFA, 2000a). The ADI is 15 times higher than the MSDI of 16 mg per capita per day. The total combined intake for the remaining substances from structural class I is approximately 1 400 µg per capita per day, which is below the threshold of 1 800 µg per person per day.

On the basis of the reported annual production volumes in Europe, the combined estimated intake as flavourings of the candidate substances assigned to structural class II is 1 300 µg per capita per day, which exceeds the threshold of concern for a compound belonging to structural class II of 540 µg per person per day. A combined intake of 1 300 µg per capita per day corresponds to 22 µg/kg bw per day. A NOAEL of 100 mg/kg bw per day has been established for one candidate substance in this group, namely methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520]. This NOAEL provides a margin of safety of more than 4 500 for the combined intake of 1 300 µg per capita per day. The Panel noted that this candidate substance [FL-no: 09.520] accounts for 60 % of this combined intake estimate.

The total combined intake from the seven candidate and 15 supporting substances from structural class II is approximately 2 200 µg per capita per day, which exceeds the threshold for structural class II substances of 540 µg per person per day. Also in the case of the total combined intake for structural class II substances, the NOAEL of 100 mg/kg bw per day, for methyl 3-oxo-2-pentyl-1-cyclopentylacetate, provides a margin of safety of 2 700 to the combined intake of 2 200 µg per capita per day, corresponding to 37 µg/kg bw per day.

The daily per capita intake of the only candidate substance from structural class III, 6-isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one [FL-no: 06.136], is 1.2 µg, which does not exceed the threshold of 90 µg per person per day. There are no supporting substances from structural class III.

9. Toxicity

9.1. Acute toxicity

Data are available for five candidate substances [FL-nos: 02.070, 02.135, 06.136, 09.520 and 09.355]. Oral median Lethal Dose (LD_{50}) values from studies in the rat range from 625 mg/kg bw to > 5 000 mg/kg bw.

Ten supporting substances [FL-nos: 02.015, 02.061, 02.062, 02.209, 07.111, 07.148, 07.176, 09.016, 09.215 and 09.216] were tested for acute toxicity in the mouse, rat, rabbit, dog and guinea pig. The LD_{50} values ranged from 930 mg/kg bw to > 5 000 mg/kg bw.

The magnitudes of the LD_{50} values indicate that the oral acute toxicity is rather low for the candidate substances and supporting substances.

The acute toxicity data are summarised in Table 14.

9.2. Subacute, subchronic, chronic and carcinogenicity studies

Only one conventional subchronic oral study has been conducted on one candidate substance [FL-no: 09.520]. For cyclohexanol [FL-no: 02.070] data were available from a study designed to investigate only peripheral neuropathy in which rats were given intraperitoneal doses of 200 mg cyclohexanol once or twice daily for up to six weeks. No effects on the peripheral nervous system were observed but the experiment was terminated early because the animals were in poor condition, there was a decrement of weight gain and two animals died prematurely. No general gross or histopathological examinations were reported and no NOAEL was established. This study was not considered applicable to the evaluation of the oral toxicity of cyclohexanol (Perbellini et al., 1981).

For methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] a study, following the current Organisation for Economic Co-operation and Development (OECD) guidelines, was performed in rats. Male and female Sprague–Dawley CD rats were given, in their diets, daily doses of 0 (basal diet), 10, 50 or 100 mg/kg bw of methyl 3-oxo-2-pentyl-1-cyclopentylacetate over a 90-day period (10 males and 10 females per dose group). No treatment-related changes were observed in mortality, expanded clinical observations, ophthalmic examination, body weight gain, body weight change, food consumption, haematology, clinical chemistry, urinalysis, organ weights and macroscopic examination. There were no treatment-related histopathological changes in the tissues from rats of any of the treatment groups. The NOAEL was therefore considered to be 100 mg/kg bw per day (the highest dose tested) (Kelly and Bolte, 2000).

Carcinogenicity studies are available for the two supporting substances, cyclohexanone and menthol [FL-nos: 07.148 and 02.015].

Cyclohexanone [FL-no: 07.148] was given to male and female rats in drinking water (doses of 3 300 and 6 500 mg/kg) and male and female mice [doses of 6 500, 13 000 and 25 000 mg/kg (only female)] for two years. A reduction in weight gain (15–20 %) was observed in all groups at the highest doses. An increase in the incidence of lymphomas was observed at a lower dose level, but it was not dose related (Lijinsky and Kovatch, 1986).

In two other studies, two doses of DL-menthol were given to rats in the diet (3 750 and 7 500 mg/kg) and mice (2 000 and 4 000 mg/kg) for 103 weeks. A small reduction in survival was seen in the treated female mice. An increase of incidence of mammary gland fibroadenomas or mammary adenocarcinomas was observed in female rats at the lower dose level, but this was not dose related. In male rats, dl-menthol was not considered toxic or carcinogenic. In mice, a small increase in the incidence of hepatocellular carcinomas was observed. However, this increase was within the normal range of tumour incidence in the historical control groups, and the authors concluded that dl-menthol was not carcinogenic in rats and mice in the performed studies (National Cancer Institute, 1979).

For five supporting substances [FL-nos: 02.015, 07.095, 07.111, 07.176 and 07.148] there are further oral subchronic toxicity data.

Repeated-dose toxicity data are summarised in Table 15.

9.3. Developmental/reproductive toxicity studies

There is a study available for one candidate substance [FL-no: 02.070], with a NOAEL of < 1 500 mg/kg bw per day. For one supporting substance [FL-no: 02.015] there are several studies.

The developmental/reproductive toxicity study is summarised in Table 16.

9.4. Genotoxicity studies

Owing to the presence of a structural alert for genotoxicity ("α,β-unsaturated carbonyl moiety") for three candidate substances [FL-nos: 07.202, 07.255 and 09.870] in the current revision of FGE.09, the genotoxicity of these substances was further assessed in FGE.212 and FGE.212Rev1. In FGE.212 (EFSA, 2009) the concern for carvyl-3-methylbutyrate [FL-no: 09.870] was alleviated and the Panel concluded that this substance could be evaluated through the Procedure. Since it was concluded in FGE.212Rev1 (EFSA CEF Panel, 2011) that, based on additional information, the concern for genotoxic potential for isophorone [FL-no: 07.126] has been alleviated, a genotoxic potential can also be ruled out for substances structurally related to isophorone (including [FL-nos: 07.202 and 07.255]). Therefore, these two substances [FL-nos: 07.202 and 07.255] can be evaluated using the Procedure.

In FGE.212Rev3 (EFSA CEF Panel, 2015) it was concluded, that, based on additional information on the supporting substance 3-methyl-2-cyclopenten-1-one [FL-no: 07.112], the concern for genotoxicity for the candidate substance [FL-no: 07.219] could be ruled out. Therefore, this candidate substance can be evaluated using the Procedure.

Owing to the presence of a structural alert for genotoxicity ("α,β-unsaturated ketone") for the candidate substance 2,6,6-trimethylcyclohex-2-en-1,4-dione [FL-no: 07.109], the genotoxicity of this substance was assessed in FGE.213 and FGE.213Rev1. In FGE.213Rev1 the concern was alleviated and the Panel concluded that this substance could be evaluated through the Procedure (EFSA CEF Panel, 2014).

Genotoxicity data are available for only three of the remaining candidate substances—cyclohexanol [FL-no: 02.070], cyclopentanol [FL-no: 02.135], methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520]—and for nine supporting substances and one structurally related substance.

Cyclohexanol [FL-no: 02.070] was not genotoxic in two Ames tests (Barsky, 1976; Haworth et al., 1983) and in an *in vivo* micronucleus assay (Gelbke, 1991), which are all considered valid studies. However, the results of the *in vivo* study are of limited relevance, because of the lack of evidence that the substance did reach the bone marrow. Inconclusive results were reported in an *in vitro* chromosomal aberration assay with human leucocytes (Collin, 1971) and negative results were reported in a dominant lethal mutations assay with *Drosophila melanogaster* (Goncharova, 1970); both studies were considered inadequate.

Cyclopentanol [FL-no: 02.135] was studied in a valid Ames test (McMahon et al., 1979). No mutagenicity was found.

A battery of *in vitro* and *in vivo* genotoxicity studies were conducted on methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520] including valid negative reverse mutation tests in *E. coli* (Wagner and Klug, 2000) and *S. typhimurium* (Thompson, 2000).

In a mouse lymphoma test on methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520], pre-dating Good Laboratory Practice (GLP), a more than twofold increase in the mutant frequency over the solvent-treated control values was found at the highest tested cytotoxic concentration of 300 µg/ml in the presence of metabolic activation, and at the two highest tested cytotoxic concentrations of 200 and 300 µg/ml in the absence of metabolic activation. Only limited documentation is provided in the study report; together with the fact that several cultures were infected and a lack of a confirmatory test, it is impossible to assess the reliability of these results (Ross and Harris, 1979).

No induction of forward mutations at the thymidine kinase (TK) locus in L5178Y mouse lymphoma cells were found in a study performed in compliance with the current OECD test guidelines, both in the absence and in the presence of metabolic activation, up to and including cytotoxic concentrations (Cifone, 2001).

Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was tested in a bone marrow micronucleus test in mice following a single intraperitoneal administration of 0, 280, 560 or 1 120 mg/kg bw in corn oil. The study was performed in compliance with the current OECD test guidelines. The two highest doses chosen induced clear signs of toxicity; slight reductions (up to 12 %) in the ratio of polychromatic erythrocytes to total erythrocytes were found, indicating that the test material had reached the target cells. No increase in micronucleated cells was found in the groups treated with the test material. The positive control induced the expected increases (Gudi and Krsmanovic, 1998).

In an Unscheduled DNA Synthesis (UDS) study, the ability of methyl 3-oxo-2-pentyl-1-cyclopentylacetate to induce DNA repair was studied in isolated rat hepatocytes after administration *in vivo*. The study was performed in compliance with the current OECD Guideline 486 (OECD, 1997c). Methyl 3-oxo-2-pentyl-1-cyclopentylacetate was administered to male Sprague–Dawley CD rats by intraperitoneal injection in doses of 333.3 and 1 000 mg/kg bw (the latter dose was the maximum tolerated dose) followed by liver perfusion at 2 or 16 hours after dosing. No marked increase in the incidence of UDS was observed at either dose level or perfusion time. Statistically significant differences were revealed in the positive control groups when compared with the negative control group and the test article (Durward, 2001).

Genotoxicity data are available for nine supporting substances [FL-nos: 02.015, 02.062, 07.045, 07.148, 07.149, 07.176, 09.027, 09.215 and 09.230].

Cyclohexanone [FL-no: 07.148], structurally related to the alicyclic ketones and secondary alcohols in this FGE, was not mutagenic in an Ames test, which was considered to be valid (Haworth et al., 1983). Negative and positive results were reported in several other *in vitro* studies at gene and chromosomal level, as well as a negative result in a sex-linked recessive lethal mutations in *D. melanogaster*. However, these studies were considered inadequate.

Menthol [FL-no: 02.015] gave negative results in an *in vitro* alkaline elution assay for detecting DNA single strand breaks in rat hepatocytes (Storer et al., 1996). With the same substance equivocal results in an *in vivo* host-mediated mutation assay were observed at high-dose levels (Food and Drug Research Laboratories, Inc., 1975) and negative results in several Ames tests, a TK+/– mouse lymphoma assay (Myhr and Caspary, 1991), sister chromatid exchange (SCE) tests in Chinese hamster ovary (CHO) cells (Ivett et al., 1989) and human lymphocytes (Murthy et al., 1991), and chromosomal aberration assays with human embryonic lung cells (Food and Drug Research Laboratories, Inc., 1975), human lymphocytes (Murthy et al., 1991) and CHO cells (Ivett et al., 1989). Negative results were also reported in two *in vivo* micronucleus (Shelby et al., 1993) and chromosomal aberration assays (Food and Drug Research Laboratories, Inc., 1975). However, the results of these studies have a limited relevance, owing to the lack of bone marrow toxicity. In addition, an *in vivo* dominant lethal assay was available, from which negative results were also obtained.

trans-Menthone [FL-no: 07.176] was genotoxic in an Ames test (Andersen and Jensen, 1984) and in a somatic mutation and recombination test (SMART) with *D. melanogaster* (Franzios et al., 1997). The observed effects were not very pronounced. Further, *trans*-menthone is easily converted to menthol, which is estimated to be, overall, negative in genotoxicity tests.

Carveol and carvyl acetate [FL-nos: 02.062 and 09.215] were tested in an Ames test at various doses from 10 to 560 µg/plate in the *S. typhimurium* strains TA97, TA98, TA100, TA1535 and TA1537 with and without S9 mix in dimethyl sulphoxide. Positive and negative controls were used. No mutagenicity was observed (Mortelmans et al., 1986).

Conclusion on genotoxicity

For five of the candidate substances [FL-nos: 07.109, 07.202, 07.219, 07.255 and 09.870] it has been concluded that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for supporting substances.

Some genotoxicity data are available for only three of the remaining candidate substances, and for these three mainly negative results were obtained. For the supporting substances, mainly negative, but also some positive results were obtained. The positive results were obtained from poorly reported tests, or tests that are difficult to interpret with respect to their relevance for genotoxicity.

Overall, the genotoxic potential of this group of flavouring substances cannot be fully assessed as it is now. However, the data available do not indicate a genotoxic potential and therefore do not preclude their evaluation via the Procedure.

Data on genotoxicity are summarised in Tables 17 (*in vitro*) and 18 (*in vivo*).

CONCLUSIONS

The present revision of FGE.09, FGE.09Rev6, includes the assessment of one additional candidate substance, trans-3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one [FL-no: 07.219], which was not included in FGE.09Rev5.

FGE.09Rev6 deals with 22 candidate substances; secondary alicyclic saturated and unsaturated alcohols, ketones, one hemiketal ester and esters containing secondary alicyclic alcohols. These flavouring substances belong to chemical groups 8, 25 and 30 of Annex I of Regulation (EC) No 1565/2000.

Two candidate substances [FL-nos: 07.203 and 07.255] possess one chiral centre and 15 substances [FL-nos: 02.075, 02.167, 06.136, 07.059, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] possess two or more chiral centres.

Fourteen candidate substances belong to structural class I, seven substances belong to structural class II and one to structural class III according to the decision tree approach presented by Cramer et al. (1978).

Fifteen of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, intakes in Europe of the 14 flavouring substances belonging to structural class I rangefrom 0.0012 to 830 µg per capita per day, intakes of the seven substances from structural class II range from 0.0085 to 530 µg per capita per day and intake of the substance from structural class III is 1.2 µg per capita per day, which are all below the threshold of concern values for structural classes I, II or III of 1 800, 540 or 90 µg per person per day, respectively. For one substance [FL-no: 09.520] from structural class II, the MSDI is 770 µg per capita per day, which is above the threshold of concern of 540 µg per person per day. For this substance a NOAEL is available, providing a sufficient margin of safety based on the MSDI approach.

The total combined intakes of candidate and supporting substances from structural classes I and II do not give rise to a safety concern.

For five candidate substances [FL-nos: 07.109, 07.202, 07.219, 07.255 and 09.870] it has been concluded that a concern for genotoxicity, indicated by the presence of a structural alert, could be ruled out based on experimental data for supporting substances. Genotoxicity data are available for only a limited number of the remaining flavouring substances in the present group and the genotoxicity cannot be assessed adequately. However, the data available do not preclude evaluation of the substances using the Procedure.

All 22 candidate substances are expected to be metabolised to innocuous products at the estimated levels of use as flavouring substances.

It was noted that where toxicity data were available they were consistent with the conclusions in the present FGE using the Procedure.

It is considered that the 22 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances based on the default MSDI approach.

In order to determine whether the conclusion for the 22 candidate substances, which have been evaluated using the Procedure, can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications, including complete purity criteria and information on identity for the materials of commerce, have been provided for all flavouring substances.

Thus, for 22 flavouring substances evaluated using the Procedure, the Panel considered that the materials of commerce would not present a safety concern at their estimated levels of intake based on the MSDI approach [FL-nos: 02.070, 02.075, 02.135, 02.167, 06.136, 07.059, 07.109, 07.202, 07.203, 07.219, 07.255, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949].

The estimated intakes for 13 candidate substances in structural class I, based on the mTAMDI approach, ranged from 420 to 63 000 µg per person per day. For six substances [FL-nos: 09.154, 09.618, 09.619, 09.843, 09.935 and 09.949], the mTAMDI is above the threshold of concern of 1 800 µg per person per day. For seven substances [FL-nos: 02.070, 02.075, 02.135, 02.167, 09.355, 09.621 and 09.870], the mTAMDI is below the threshold. The mTAMDIs of five substances assigned to structural class II range from 1 600 to 8 700 µg per person per day, which are above the threshold of concern for structural class II substances of 540 µg per person per day. The mTAMDI estimates for one substance from structural class II [FL-no: 07.255] and for the one candidate substance in class III [FL-no: 06.136] are 320 and 0.075 µg per person per day, respectively, which are below the thresholds of their structural classes (540 and 90 µg per person per day). For all substances with mTAMDI values below their structural class thresholds, the Panel noted that they have been evaluated via the A-side of the Procedure.

For one flavouring substance [FL-no: 09.929] from structural class I and one flavouring substance [FL-no: 07.219] from structural class II, use levels are missing and an mTAMDI cannot be calculated for these two substances.

In conclusion, for 11 candidate substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 07.219 09.154, 09.520, 09.618, 09.619, 09.843, 09.935 and 09.949], for which the mTAMDIs are above the thresholds for their structural class, and for another two substances [FL-nos: 07.219 and 09.929], for which use levels are missing, further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
bw	body weight
CAS	Chemical Abstract Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
GLP	Good Laboratory Practice
FLAVIS (FL)	Flavour Information System (database)
ID	Identity
IOFI	International Organization of the Flavor Industry
IP	Intraperitoneal
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50 %; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NMR	Nuclear magnetic resonance
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
SCE	sister chromatid exchange
SCF	Scientific Committee on Food
SMART	somatic mutation and recombination test
TAMDI	Theoretical Added Maximum Daily Intake
TK	Thymidine Kinase
UDS	Unscheduled DNA Synthesis
WHO	World Health Organization

APPENDICES

Appendix A. Summary of safety evaluation

Table 6: Summary of safety evaluation applying the procedure

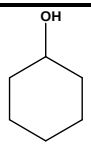
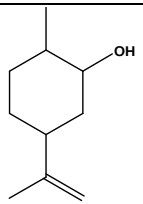
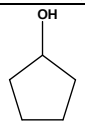
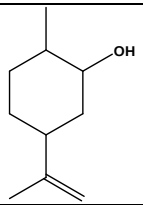
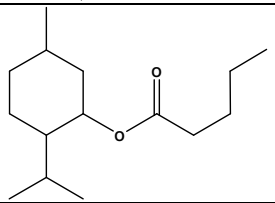
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg per capita per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d)(e)]	Outcome on the material of commerce [(f)(g)(h)]	Evaluation remarks
02.070	Cyclohexanol		3.7	Class I A3: Intake below threshold	d	f	
02.075	(1*R*,2*S*,5*S*)-neo-Dihydrocarveol		2.4	Class I A3: Intake below threshold	d	f	
02.135	Cyclopentanol		0.012	Class I A3: Intake below threshold	d	f	
02.167	(1*R*,2*R*,5*S*)-Isodihydrocarveol		2.4	Class I A3: Intake below threshold	d	f	
09.154 1852	Menthyl valerate		1	Class I A3: Intake below threshold	d	f	

Table 6: Summary of safety evaluation applying the procedure

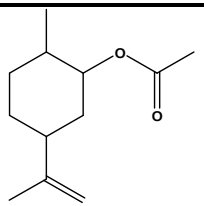
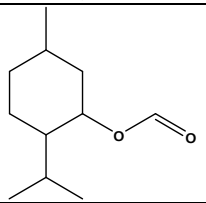
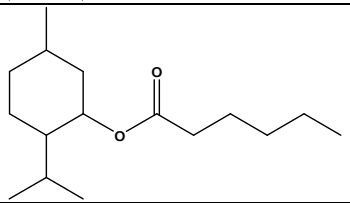
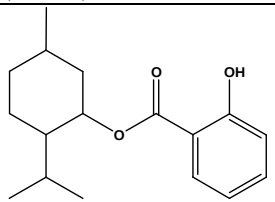
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg per capita per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d)(e)]	Outcome on the material of commerce [(f)(g)(h)]	Evaluation remarks
09.355	neo-Dihydrocarvyl acetate		0.012	Class I A3: Intake below threshold	d	f	
09.618	Menthyl formate		0.73	Class I A3: Intake below threshold	d	f	
09.619	(1*R*,2*S*,5*R*)-Menthyl hexanoate		0.37	Class I A3: Intake below threshold	d	f	
09.621	(1*R*,2*S*,5*R*)-Menthyl salicylate		0.012	Class I A3: Intake below threshold	d	f	

Table 6: Summary of safety evaluation applying the procedure

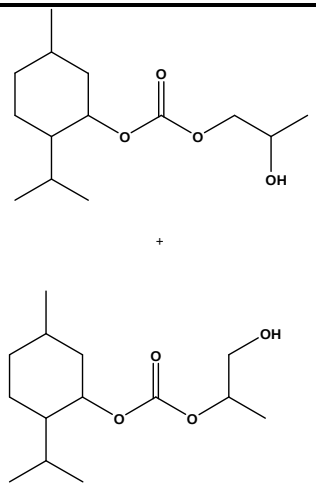
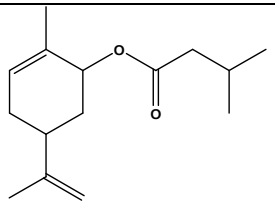
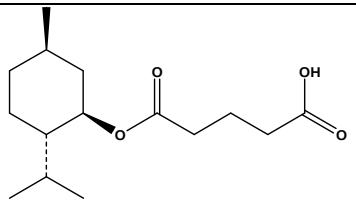
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg per capita per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d)(e)]	Outcome on the material of commerce [(f)(g)(h)]	Evaluation remarks
09.843	Menthol 1- and 2-propylene glycol carbonate		830 380	Class I A3: Intake below threshold	d	f	
09.870	Carvyl-3-methylbutyrate		0.0012	Class I A3: Intake below threshold	d	f	Evaluated in FGE.212, genotoxic concern could be ruled out
09.929	L-Monomenthyl glutarate		110	Class I A3: Intake below threshold	d	f	

Table 6: Summary of safety evaluation applying the procedure

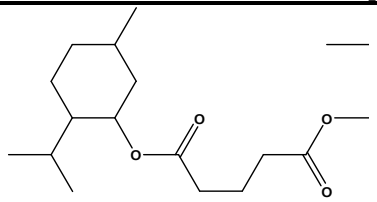
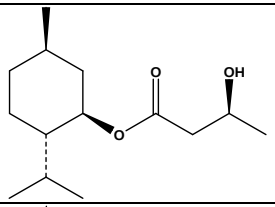
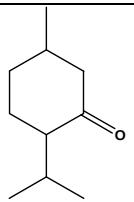
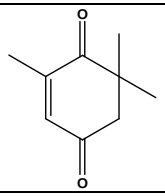
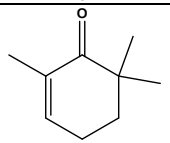
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg per capita per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d)(e)]	Outcome on the material of commerce [(f)(g)(h)]	Evaluation remarks
09.935	Dimenthyl glutarate		30	Class I A3: Intake below threshold	d	f	
09.949	L-Menthyl (*S*)-3-hydroxybutyrate		37	Class I A3: Intake below threshold	d	f	
07.059	*p*-Menthan-3-one		530 2500	Class II A3: Intake below threshold	d	f	
07.109 1857	2,6,6-Trimethylcyclohex-2-en-1,4-dione		50	Class II A3: Intake below threshold	d	f	Evaluated in FGE.213Rev1, genotoxicity concern could be ruled out
07.202	2,6,6-Trimethylcyclohex-2-en-1-one		0.12	Class II A3: Intake below threshold	d	f	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out

Table 6: Summary of safety evaluation applying the procedure

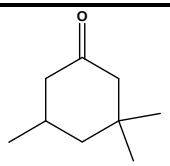
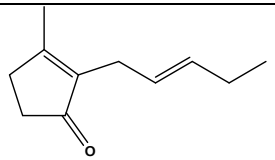
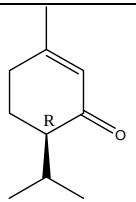
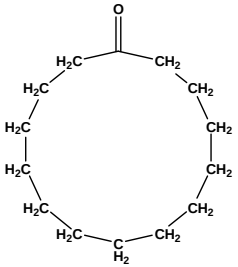
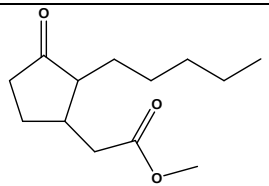
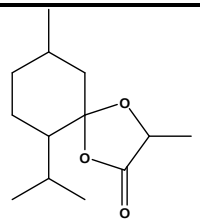
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg per capita per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d)(e)]	Outcome on the material of commerce [(f)(g)(h)]	Evaluation remarks
07.203	3,3,5-Trimethylcyclohexan-1-one		0.0085	Class II A3: Intake below threshold	d	f	
07.219	trans-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one		4.7	Class II A3: Intake below threshold	d	f	Evaluated in FGE.212Rev3, genotoxicity concern could be ruled out
07.255 1856	l-Piperitone		12	Class II A3: Intake below threshold	d	f	Evaluated in FGE.212Rev1, genotoxic concern could be ruled out
07.207	Cyclotetradecanone		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Flavour Industry (EFFA, 2009)
09.520 1898	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate		770	Class II A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	d	f	

Table 6: Summary of safety evaluation applying the procedure

FL-no	EU Register name	Structural formula	MSDI [(a)] (µg per capita per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d)(e)]	Outcome on the material of commerce [(f)(g)(h)]	Evaluation remarks
06.136 1859	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one		1.2	Class III A3: Intake below threshold	d	f	

(a): EU MSDI: Amount added to food as flavour in (kg/year) × 10E9/(0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg per capita per day.
(b): Thresholds of concern: Class I = 1 800 µg per person per day, Class II = 540 µg per person per day, Class III = 90 µg per person per day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).
(g): Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
(h): No conclusion can be drawn because of the lack of information on the purity of the material of commerce.

Table 7: Evaluation status of hydrolysis products of candidate esters in FGE.09Rev6

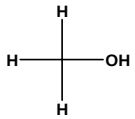
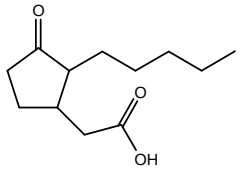
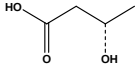
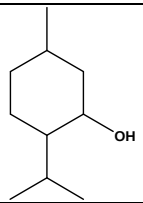
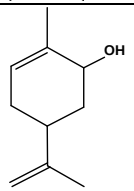
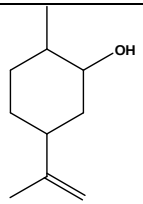
FL-no	EU Register name JECFA no	Structural formula	SCF status [a] JECFA status [b] CoE status [c] EFSA status	Structural class [d] Procedure path (JECFA) [e]	Comments
Not in Register	Methanol		Not evaluated as flavouring substance		Not a Register substance.
Not in Register	3-Oxo-2-pentyl-1-cyclopentyl acetic acid		Not evaluated as flavouring substance		Not a Register substance.
Not in Register	(*S*)-3-Hydroxybutyric acid		Not evaluated as flavouring substance		Not a Register substance.
02.015	Menthol 427		No safety concern (JECFA, 2000a) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	NOAEL: 380 mg/kg bw/day
02.062	Carveol 381		No safety concern (JECFA, 2000a) Category B (CoE, 1992)	Class I A3: Intake below threshold	
02.075	(1*R*,2*S*,5*S*)-neo-Dihydrocarveol		Category B (CoE, 1992) FGE.09	Class I A3: Intake below threshold	

Table 7: Evaluation status of hydrolysis products of candidate esters in FGE.09Rev6

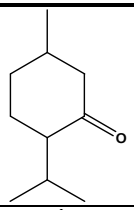
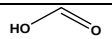
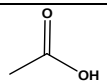
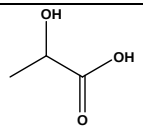
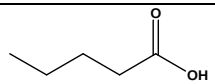
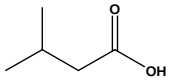
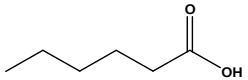
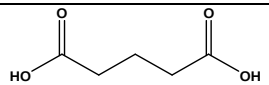
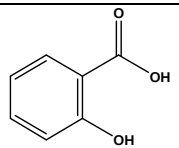
FL-no	EU Register name JECFA no	Structural formula	SCF status [a] JECFA status [b] CoE status [c] EFSA status	Structural class [d] Procedure path (JECFA) [e]	Comments
07.059	*p*-Menthan-3-one		Category B (CoE, 1992) FGE.09	Class II A3: Intake below threshold	
08.001	Formic acid 79		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Deleted (CoE, 1992)	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.004	Lactic acid 930		No safety concern (JECFA, 2002a) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.007	Valeric acid 90		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake below threshold	
08.008	3-Methylbutyric acid 259		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake below threshold	

Table 7: Evaluation status of hydrolysis products of candidate esters in FGE.09Rev6

FL-no	EU Register name JECFA no	Structural formula	SCF status [(a)] JECFA status [(b)] CoE status [(c)] EFSA status	Structural class [(d)] Procedure path (JECFA) [(e)]	Comments
08.009	Hexanoic acid 93		Category 1 (SCF, 1995) No safety concern (JECFA, 1999b) Category A (CoE, 1992)	Class I A3: Intake above threshold, A4: Endogenous	
08.082	Glutaric acid		FGE.10	Class I A3: Intake below threshold	
08.112	Salicylic acid 958		No safety concern (JECFA, 2002a)	Class I A3: Intake below threshold	

(a): Category 1: Considered safe in use. Category 2: Temporarily considered safe in use. Category 3: Insufficient data to provide assurance of safety in use. Category 4: Not acceptable because of evidence of toxicity.
(b): No safety concern at estimated levels of intake.
(c): Category A: Flavouring substance which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.
(d): Threshold of concern: Class I = 1 800 µg per person per day, Class II = 540 µg per person per day, Class III = 90 µg per person per day.
(e): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

SUPPORTING SUBSTANCES SUMMARY

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

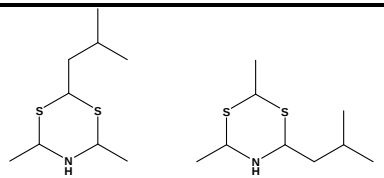
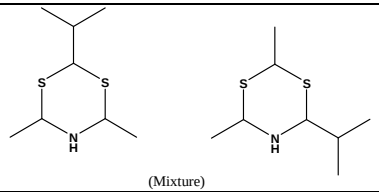
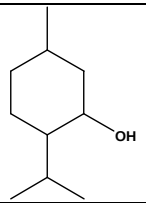
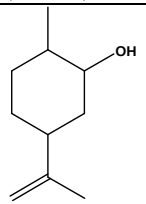
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (µg per capita per day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
	2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	 (Mixture)	3781 101517-87-7 and 101517-86-6	1046 JECFA specification (JECFA, 2002c)	0.1	No safety concern	Not in the EU Register
	2-Isopropyl-4,6-dimethyl 2,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	 (Mixture)	3782 104691-41-0 and 104691-40-9	1047 JECFA specification (JECFA, 2002c)	ND	No safety concern	Not in the EU Register
02.015	Menthol		63 89-78-1	427 JECFA specification (JECFA, 1998)	16 000	No safety concern (JECFA, 2000a) Category A (CoE, 1992)	ADI: 0–4 mg/kg bw (JECFA, 2000a)
02.061	Dihydrocarveol		2379 2025 619-01-2	378 JECFA specification (JECFA, 1998)	0.37	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

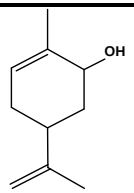
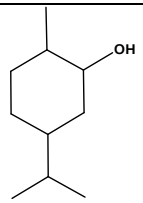
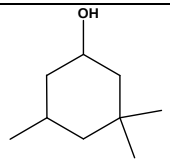
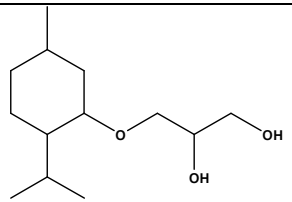
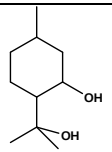
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [(a)] (µg per capita per day)	SCF status [(b)] JECFA status [(c)] CoE status [(d)]	Comments
02.062	Carveol		2247 2027 99-48-9	381 JECFA specification (JECFA, 1998)	9.5	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
02.071	*p*-Menthan-2-ol		3562 2228 499-69-4	376 JECFA specification (JECFA, 2000b)	0.012	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
02.209	3,3,5-Trimethylcyclohexan-1-ol		3962 116-02-9	1099 JECFA specification (JECFA, 2002c)	0.12	No safety concern (JECFA, 2002b)	JECFA name: 3,3,5-Trimethyl cyclohexanol
02.224	3-(1-Menthoxy)propane-1,2-diol		3784 87061-04-9	1408 JECFA specification (JECFA, 2005a)	4.1	No safety concern (JECFA, 2005b)	JECFA name: 3-L-Menthoxypropane-1,2-diol
02.246	*p*-Menthane-3,8-diol		4053 42822-86-6	1416 JECFA specification (JECFA, 2005a)	39	No safety concern (JECFA, 2005b)	

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

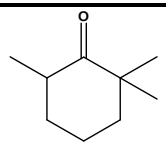
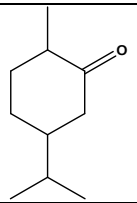
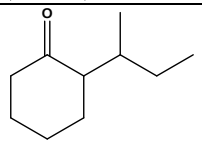
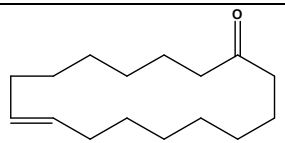
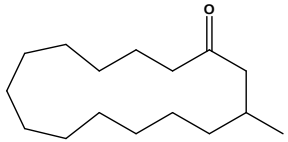
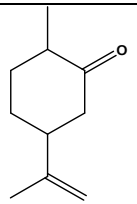
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [(a)] (μg per capita per day)	SCF status [(b)] JECFA status [(c)] CoE status [(d)]	Comments
07.045	2,2,6-Trimethylcyclohexanone		3473 686 2408-37-9	1108 JECFA specification (JECFA, 2002c)	2.1	No safety concern (JECFA, 2002b) Category B (CoE, 1992)	
07.092	*p*-Menthan-2-one		3176 11128 499-70-7	375 JECFA specification (JECFA, 1998)	0.012	No safety concern (JECFA, 2000a)	
07.095	2-(sec-Butyl)cyclohexanone		3261 11044 14765-30-1	1109 JECFA specification (JECFA, 2002c)	5.1	No safety concern (JECFA, 2002b)	
07.110	Cycloheptadec-9-en-1-one		3425 11744 542-46-1	1401 JECFA specification (JECFA, 2005a)	0.24	No safety concern (JECFA, 2005b)	
07.111	3-Methylcyclopentadecan-1-one		3434 11135 541-91-3	1402 JECFA specification (JECFA, 2005a)	0.37	No safety concern (JECFA, 2005b)	JECFA name: 3-Methyl-1-cyclopentadecanone.
07.128	Dihydrocarvone		3565 11703 7764-50-3	377 JECFA specification (JECFA, 2000b)	0.012	No safety concern (JECFA, 2000a)	

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

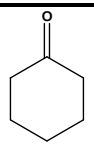
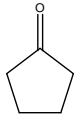
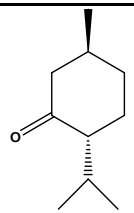
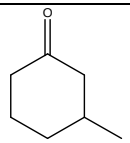
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (μg per capita per day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
07.148	Cyclohexanone		3909 11047 108-94-1	1100 JECFA specification (JECFA, 2002c)	0.12	No safety concern (JECFA, 2002b)	
07.149	Cyclopentanone		3910 11050 120-92-3	1101 JECFA specification (JECFA, 2002c)	0.018	No safety concern (JECFA, 2002b)	
07.176	trans-Menthone	 (-)menthone shown	2667 2035 89-80-5	429 JECFA specification (JECFA, 1998)	890	No safety concern (JECFA, 2000a)	JECFA name: Menthone. CAS No in register refers to cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2*R*,5*S*)-rel-
07.180	3-Methylcyclohexanone		3947 591-24-2	1103 JECFA specification (JECFA, 2002c)	0.12	No safety concern (JECFA, 2002b)	

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

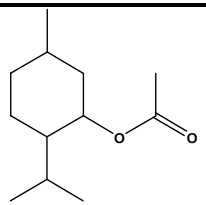
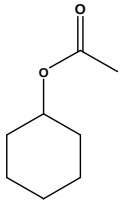
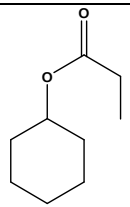
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (μg per capita per day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
09.016	Menthyl acetate		2668 206 29066-34-0	431 JECFA specification (JECFA, 1998)	270	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	JECFA evaluated menthyl acetate (CAS No 16409-45-3, which does not specify isomer). CAS No in register replaced by 89-48-5, which refers to Cyclohexanol, 5-methyl-2-(1-methylethyl)-, acetate, (1*R*,2*S*,5*R*) (SciFinder)
09.027	Cyclohexyl acetate		2349 217 622-45-7	1093 JECFA specification (JECFA, 2002c)	12	No safety concern (JECFA, 2002b) Category B (CoE, 1992)	
09.140	Cyclohexyl propionate		2354 421 6222-35-1	1097 JECFA specification (JECFA, 2002c)	0.012	No safety concern (JECFA, 2002b) Category B (CoE, 1992)	

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

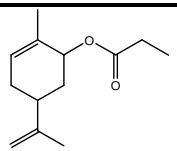
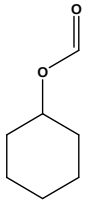
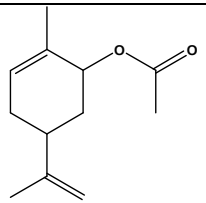
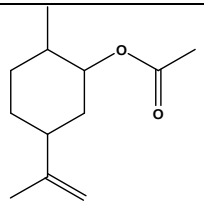
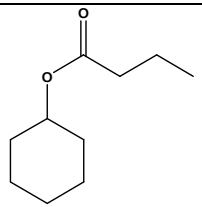
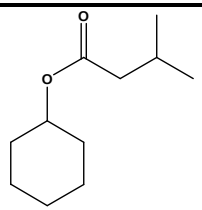
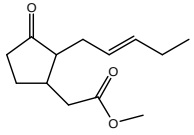
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (μg per capita per day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
09.143	Carvyl propionate		2251 424 97-45-0	383 JECFA specification (JECFA, 2000b)	0	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.160	Cyclohexyl formate		2353 498 4351-54-6	1095 JECFA specification (JECFA, 2002c)	0.012	No safety concern (JECFA, 2002b) Category B (CoE, 1992)	
09.215	Carvyl acetate		2250 2063 97-42-7	382 JECFA specification (JECFA, 1998)	4	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.216	Dihydrocarvyl acetate		2380 2064 20777-49-5	379 JECFA specification (JECFA, 1998)	9.7	No safety concern (JECFA, 2000a) Category B (CoE, 1992)	
09.230	Cyclohexyl butyrate		2351 2082 1551-44-6	1094 JECFA specification (JECFA, 2002c)	0.89	No safety concern (JECFA, 2002b) Category B (CoE, 1992)	

Table 8: Summary of safety evaluation of supporting substances performed by the JECFA

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [(a)] (µg per capita per day)	SCF status [(b)] JECFA status [(c)] CoE status [(d)]	Comments
09.464	Cyclohexyl isovalerate		2355 459 7774-44-9	1096 JECFA specification (JECFA, 2002c)	0.28	No safety concern (JECFA, 2002b) Category B (CoE, 1992)	
09.521	Methyl 3-oxo-2-pent-2-enyl-1-cyclopentylacetate		3410 10821 39924-52-2	1400 JECFA specification (JECFA, 2005a)	26	No safety concern (JECFA, 2005b)	JECFA evaluated methyl jasmonate (CAS No 1211-29-6). (R)- or (S)- nor (E)- or (Z)- not specified by register CAS No

ND, No intake data reported.
(a): EU MSDI: Amount added to food as flavouring substance in (kg / year) × 10E9 / (0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita per day.
(b): Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.
(c): No safety concern at estimated levels of intake.
(d): Category A: Flavouring substance which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

Appendix B. Procedure for the safety evaluation

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000, named the "Procedure", is shown in schematic form in Figure B.1. The Procedure is based on the Opinion of the SCF expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation Procedure developed by JECFA at its 44th, 46th and 49th meetings (JECFA, 1995, 1996, 1997, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure–activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern of 1 800, 540 or 90 µg per person per day for classes I, II and III, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- Can the flavourings be predicted to be metabolised to innocuous products[12] (Step 2)?
- Do their exposures exceed the threshold of concern for the structural class (Steps A3 and B3)?
- Are the flavourings or their metabolites endogenous[13] (Step A4)?
- Does a NOAEL exist on the flavourings or on structurally related substances (Steps A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[12] "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

[13] "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

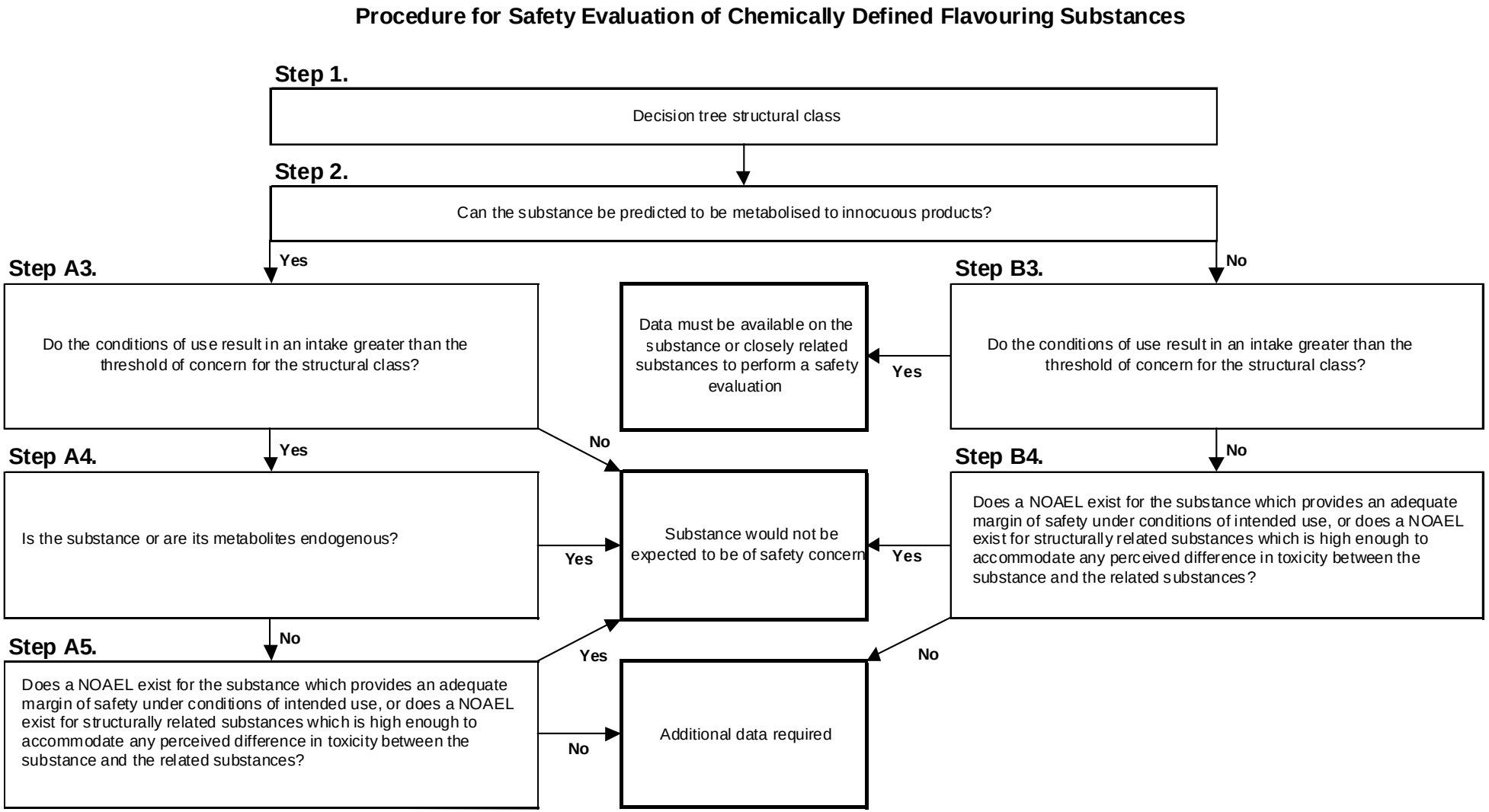


Figure 1: Procedure for safety evaluation of chemically defined flavouring substances

Appendix C. Use levels/mTAMDI

C.1 Normal and maximum use levels

For each of the 18 Food categories (Table 9) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level". According to the Industry the "normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004).

Table 9: Food categories according to Commission Regulation (EC) No 1565/2000

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds
05.0	Confectionery
06.0	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excluding dairy products
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat)—foods that could not be placed in categories 01.0–15.0

The "normal and maximum use levels" are provided by the Flavour industry (Burdock, 1995; EFFA, 2003a, 2007; Flavour Industry, 2004, 2006a, b, 2007, 2010a, b) for 20 of the 22 candidate substances in the present flavouring group (Table 10).

Table 10: Normal and maximum use levels (mg/kg) for the candidate substances FGE.09Rev5 (Burdock, 1995; EFFA, 2003a, 2007; Flavour Industry, 2004, 2006a, 2006b, 2007, 2010a, 2010b).

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
02.070	3 15	2 10	3 15	2 10	–	4 20	2 10	5 25	1 5	1 5	–	–	2 10	3 15	2 10	4 20	5 25	2 10
02.075	3 15	2 10	3 15	2 10	–	4 20	2 10	5 25	1 5	1 5	–	–	2 10	3 15	2 10	4 20	5 25	2 10
02.135	3 15	2 10	3 15	2 10	–	4 20	2 10	5 25	1 5	1 5	–	–	2 10	3 15	2 10	4 20	5 25	2 10
02.167	3 15	2 10	3 15	2 10	–	4 20	2 10	5 25	1 5	1 5	–	–	2 10	3 15	2 10	4 20	5 25	2 10
06.136	0.0001 0.0005	0.0001 0.0001	0.0001 0.0008	–	–	0.001 0.005	0.0001 0.0005	0.0001 0.0005	–	–	–	–	–	0.0001 0.0005	0.0001 0.0005	0.0001 0.0008	–	0.0001 0.0005
07.059	– –	– –	15.32 22.99	–	–	33.27 52.97	– –	47.89 68.1	–	–	–	–	–	– –	4.22 5.86	0.87 2.59	–	–
07.109	7 35	2 10	3 15	2 10	–	4 20	– –	5 25	1 5	1 5	–	–	2 10	3 15	2 10	4 20	5 25	2 10
07.202	3 15	2 10	3 15	2 10	–	4 20	2 10	5 25	1 5	1 5	–	–	2 10	3 15	2 10	4 20	5 25	2 10
07.203	3 15	2 10	3 15	2 10	–	4 20	2 10	5 25	1 5	1 5	–	–	2 10	3 15	2 10	4 20	5 25	2 10
07.255	0.5 5	– –	0.2 2	–	–	0.5 5	– –	1 10	–	–	–	–	0.2 2	–	0.2 1	0.2 2	5 50	0.2 2
09.154	7 35	5 25	10 50	7 35	–	10 50	5 25	10 50	2 10	2 10	–	–	5 25	10 50	5 25	10 50	20 100	5 25
09.355	3 15	2 10	3 15	2 10	–	4 20	2 10	5 25	1 5	1 5	–	–	2 10	3 15	2 10	4 20	5 25	2 10
09.520	7 35	5 25	10 50	7 35	–	10 50	5 25	10 50	2 10	2 10	–	–	5 25	10 50	5 25	10 50	20 100	5 25
09.618	7 35	5 25	10 50	7 35	–	10 50	5 25	10 50	2 10	2 10	–	–	5 25	10 50	5 25	10 50	20 100	5 25
09.619	7 35	5 25	1 50	7 35	–	10 50	5 25	10 50	2 10	2 10	–	–	5 25	10 50	5 25	10 50	20 100	5 25

Table 10: Normal and maximum use levels (mg/kg) for the candidate substances FGE.09Rev5 (Burdock, 1995; EFFA, 2003a, 2007; Flavour Industry, 2004, 2006a, 2006b, 2007, 2010a, 2010b).

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
09.621	0.5	0.2	0.5	0.4	–	1	0.2	2	0.2	0.2	–	–	0.3	0.5	0.2	1	2	0.4
	2.5	1	2.5	2	–	5	1	10	1	1	–	–	1.5	2.5	1	5	10	2
09.843	200	–	100	–	–	500	15	60	–	–	–	–	25	–	30	100	25	–
	800	–	400	–	–	2 000	60	250	–	–	–	–	100	–	120	400	100	–
09.870	3	2	3	2	–	4	2	5	1	1	–	–	2	3	–	4	5	2
	15	10	15	10	–	20	10	25	5	5	–	–	10	15	–	20	25	10
09.935	1	1	10	1	1	100	–	10	1	1	–	–	1	–	100	100	1	1
	15	15	150	15	15	1 500	–	150	15	15	–	–	15	–	1 500	1 500	15	15
09.949	30	–	10	20	–	50	5	20	–	–	–	–	10	–	10	10	30	10
	150	–	50	100	–	200	20	100	–	–	–	–	30	–	50	50	150	30

The candidate substances [FL-nos: 07.059, 09.843 and 09.949] are also used in chewing gum, which is not covered by any of the above food categories. The normal/maximum use levels for these substances in chewing gum are reported to be 14.34/14.34 mg/kg [FL-no: 07.059], 5 000/20 000 mg/kg [FL-no: 09.843] and 500/1 000 mg/kg [FL-no: 09.949]. Under the assumptions that all of the flavouring substances are released from the chewing gum and that the intake estimate is 2 g chewing gum per day, the calculation of the mTAMDI of the candidate substance based on the 16 food categories and the use of chewing gum sum up to 8 700, 63 000 and 10 600 µg per person per day, respectively. These figures are presented in Tables 5 and 9.

C.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table 11. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table 11: Estimated amount of flavourable foods, beverages and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g per day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by the Flavour Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 and reported by the Flavour Industry in the following way (see Table 12):

- Beverages correspond to food category 14.1.
- Foods correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13 and/or 16.
- Exception a corresponds to food categories 5 and 11.
- Exception b corresponds to food category 15.
- Exception c corresponds to food category 14.2.
- Exception d corresponds to food category 12.
- Exception e corresponds to others, e.g. chewing gum.

Table 12: Distribution of the 16 food categories listed in Commission Regulation (EC) No 1565/2000 into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation 1565/2000	**Distribution of the seven SCF food categories**		
Key	**Food category**	**Food**	**Beverages**	**Exceptions**
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic (“soft”) beverages, excluding dairy products		Beverages	
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat)—foods that could not be placed in categories 01.0–15.0	Food		

The mTAMDI values (see Table 13) are presented for each of the flavouring substances in the present flavouring group, for which the Flavour Industry has provided use and use levels (Burdock, 1995; EFFA, 2003a, 2007; Flavour Industry, 2004, 2006a, b, 2007, 2010a, b). The mTAMDI values are only given for highest reported normal use levels (see Table 13).

Table 13: Estimated intakes based on the mTAMDI approach.

FL-no	EU Register name	mTAMDI (µg per person per day)	Structural class	Threshold of concern (µg per person per day)
02.070	Cyclohexanol	1 600	Class I	1 800
02.075	(1*R*,2*S*,5*S*)-neo-Dihydrocarveol	1 600	Class I	1 800
02.135	Cyclopentanol	1 600	Class I	1 800
02.167	(1*R*,2*R*,5*S*)-Isodihydrocarveol	1 600	Class I	1 800
09.154	Menthyl valerate	3 900	Class I	1 800
09.355	neo-Dihydrocarvyl acetate	1 600	Class I	1 800
09.618	Menthyl formate	3 900	Class I	1 800
09.619	(1*R*,2*S*,5*R*)-Menthyl hexanoate	3 900	Class I	1 800
09.621	(1*R*,2*S*,5*R*)-Menthyl salicylate	420	Class I	1 800
09.843	Menthol 1- and 2-propylene glycol carbonate	63 000	Class I	1 800
09.870	Carvyl-3-methylbutyrate	1 000	Class I	1 800
09.929	L-Monomenthyl glutarate		Class I	1 800
09.935	Dimenthyl glutarate	38 000	Class I	1 800
09.949	L-Menthyl (*S*)-3-hydroxybutyrate	10 600	Class I	1 800
07.059	p-Menthan-3-one	8 700	Class II	540
07.109	2,6,6-Trimethylcyclohex-2-en-1,4-dione	1 900	Class II	540
07.202	2,6,6-Trimethylcyclohex-2-en-1-one	1 600	Class II	540
07.203	3,3,5-Trimethylcyclohexan-1-one	1 600	Class II	540
07.219	trans-3-Methyl-2-(2-pentenyl)–2-cyclopenten-1-one		Class II	540
07.255	l-Piperitone	320	Class II	540
09.520	Methyl 3-oxo-2-pentyl-1-cyclopentylacetate	3 900	Class II	540
06.136	6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one	0.075	Class III	90

Appendix D. Metabolism

D.1 Absorption, distribution and elimination

The candidate substances of secondary alicyclic saturated and unsaturated alcohols, ketones and esters of the present flavouring group evaluation are expected to be rapidly absorbed from the gastrointestinal tract. Supporting substances evaluated by the JECFA sustain this view (JECFA, 1999a, 2003).

D.2 Biotransformation

The candidate substances are expected to be metabolised through several alternative metabolic pathways. Depending on their chemical structure, the possible metabolic reactions are the following:

D.2.1 Ester hydrolysis

D.2.2 Reduction of ketone groups and oxidation of alcohol groups

D.2.3 Oxidation of alkyl groups on alkyl substituted alicyclic ketones and alcohols

D.2.4 Metabolism to glucuronides

D.2.5 Metabolism to sulphates

D.2.1. Ester hydrolysis

The esters included in this FGE are expected to be hydrolysed enzymatically to carboxylic acids and alcohols via carboxylesterases found in most tissues throughout the body, the most important of which are the β-esterases (Heymann, 1980). For the one hemiketal ester [FL-no: 06.136] hydrolysis to the corresponding cyclic ketone, p-menthan-3-one [FL-no: 07.059] and lactic acid [FL-no: 08.004] is expected.

The supporting substances, menthyl acetate [FL-no: 09.016] and dihydrocarvyl acetate [FL-no: 09.216] were previously evaluated by JECFA (1999a), but no metabolism studies were available for these supporting substances, structurally related to the candidate substances menthyl valerate, neo-dihydrocarvyl acetate, menthyl formate and menthyl hexanoate [FL-nos: 09.154, 09.355, 09.618 and 09.619]. The JECFA evaluation was based on a study demonstrating about 75 % and 85 % hydrolysis of *1*-menthol propylene glycol carbonate and *1*-menthol ethylene glycol carbonate, respectively, after four hours in liver homogenate. Less than 20 % of these two substances were hydrolysed in gastric juice and intestinal fluid (Emberger, 1994a, b). More than 80 % of a radioactively labelled mandelic acid of 3,3,5-trimethylcyclohexanol, a cyclohexyl ester structurally related to the candidate substance menthyl salicylate [FL-no: 09.621], was hydrolysed after 15 minutes of incubation with rat hepatic microsomes (White et al., 1990).

Based on these data, it is anticipated that candidate esters and the one hemiketal ester [FL-nos: 06.136, 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949], after intestinal absorption are hydrolysed to the corresponding alcohols/ketone and their corresponding carboxylic acids (see Table 7). The simple mono- and di-carboxylic acids [FL-nos: 08.001, 08.002, 08.004, 08.007, 08.008, 08.009 and 08.082] and 3-hydroxybutyric acid are expected to be completely metabolised through common routes of biotransformations. The acids salicylic acid [FL-no: 08.112] and 3-oxo-2-pentyl-1-cyclopentyl acetic acid (formed from [FL-no: 09.520]) are anticipated to be conjugated and excreted with the urine.

D.2.2. Reduction of ketone groups and oxidation of alcohol groups

Seven of the candidate substances [FL-nos: 07.059, 07.109, 07.202, 07.203, 07.219, 07.255 and 09.520] contain a ketone group, which may be metabolically reduced to a hydroxyl group. This may also be expected for the hemiketal ester [FL-no: 06.136] after hydrolysis to ketone.

Incubation of human liver microsomes with the supporting substance trans-menthone resulted in formation of two metabolites. The major metabolite was a reduction product, (+)-neomenthol and a hydroxylation product, 7-hydroxymenthone was a minor metabolite (Miyazawa and Nakanishi, 2006)

Metabolism of the supporting substance carveol [FL-no: 02.062], the hydrolysis product of carvyl-3-methylbutyrate [FL-no: 09.870], was studied *in vitro*. (+)-Carveol and (+)-carvone were incubated with liver microsomes from dogs, rabbits, guinea pigs, mice, rats, monkeys and humans. (+)-Carveol was oxidised to (+)-carvone by liver microsomes of dogs, rabbits and guinea pigs, but not by those of humans, monkeys, rats and mice. On the other hand, the (+)-carvone was reduced to (+)-carveol by liver microsomes of all animals examined. These results suggest a species-specific metabolism of (+)-carveol, and shows that carveol is not converted to carvone in the liver of humans (Shimada et al., 2002).

In vivo metabolism of *l*-menthol was studied in adult male rats by giving the rats 800 mg/kg bw *l*-menthol solved in 1 % methyl cellulose solution by gavage every day for 20 days. Control rats were given vehicle only. The following metabolites of *l*-menthol were found in the urine: *p*-menthane-3,8-diol, *p*-menthane-3,9-diol, 3,8-oxy-*p*-menthane-7-carboxylic acid and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid. The main urinary metabolites were *p*-menthane-3,9-diol and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid. Menthone was not detected (Madyastha and Srivatsan, 1988).

D.2.3. Oxidation of alkyl groups on alkyl substituted alicyclic ketones and alcohols

Oxidation of alkyl groups have been observed for menthol and for the hydrolysis product of the candidate esters neo-dihydrocarvyl acetate [FL-no: 09.355] and menthyl formate [FL-no: 09.618], and for the candidate substance 3,3,5-trimethylcyclohexan-1-one [FL-no: 07.203] (Truhaut et al., 1970; Yamaguchi et al., 1994).

D.2.4. Metabolism to glucuronides

The hydrolysis product menthol, as such or after the oxidation of the alkyl ring substituents, is mainly conjugated with glucuronic acid and excreted via the bile in rats. Low levels of oxidation products were found in the urine, but no unchanged menthol was detected in the urine, faeces or bile after oral administration of radioactive labelled menthol (Yamaguchi et al., 1994).

The candidate substances isodihydrocarveol and neo-dihydrocarveol [FL-nos: 02.167 and 02.075] are also anticipated to be conjugated with glucuronic acid, since dihydrocarveol after application by gavage to rabbits was found in the urine as the glucuronide (Hämäläinen, 1912; JECFA, 1999a). However, dihydrocarveol was also found to be excreted unchanged (Fischer and Bielig, 1940; JECFA, 1999a). In rabbits, carvone is reduced to yield carveol, which then is converted to the glucuronic acid conjugate and excreted in the urine (Fischer and Bielig, 1940). Carveol, the hydrolysis product of carvyl-3-methylbutyrate [FL-no: 09.870] is therefore anticipated to be conjugated with glucuronic acid and excreted in the urine.

Salicylic acid (resulting from hydrolysis of menthyl salicylate [FL-no: 09.621]) is excreted either unchanged or as salicyluric acid and salicylic glucuronide (Vree et al., 1994).

The candidate alicyclic ketones (p-menthan-3-one [FL-no: 07.059], 2,6,6-trimethylcyclohex-2-en-1-one [FL-no: 07.202], 3,3,5-trimethylcyclohexan-1-one [FL-no: 07.203], trans-3-methyl-2-(2-pentenyl)-2- cyclopenten-1-one [FL-no: 07.219], *l*-piperitone [FL-no: 07.255] and methyl 3-oxo-2-pentyl-1-cyclopentylacetate [FL-no: 09.520]) are anticipated to be reduced to the corresponding secondary alcohols. These secondary alcohols and the candidate secondary alcohols cyclohexanol [FL-no: 02.070], neo-dihydrocarveol [FL-no: 02.075], isodihydrocarveol [FL-no: 02.167] and cyclopentanol [FL-no: 02.135] are mainly excreted as conjugates with glucuronic acid. Studies in rabbits with the supporting substance cyclohexanone [FL-no: 07.148] and with cyclopentanone and cycloheptanone show that 50–70 % of these substances are reduced to the corresponding alcohols (the candidate substances cyclohexanol [FL-no: 02.070] and cyclopentanol [FL-no: 02.135] and

cycloheptanol), which are conjugated with glucuronic acid and excreted (Elliott et al., 1959; James and Waring, 1971). Workers employed in a shoe factory were exposed to small amounts of cyclohexane in the air. Cyclohexanol and cyclohexanone were found in the urine of these workers, indicating that the same metabolic pathways are also found in humans (Governa et al., 1987). A recent study in humans shows that the main metabolite in urine after cyclohexanone or cyclohexanol exposure is not cyclohexanol-glucuronide as in rabbit and rats, but 1,2-cyclohexanediol-glucuronide (Mráz et al., 1994, 1998).

When the candidate substance 3,3,5-trimethylcyclohexan-1-one [FL-no: 07.203] was given to rats and rabbits glucuronides of 3,5,5-trimethylcyclohexanol were detected in the urine (Truhaut et al., 1979).

D.2.5. Metabolism to sulphates

A small fraction of the two candidate substances, cyclopentanol and cyclohexanol [FL-nos: 02.135 and 02.070], is anticipated to be conjugated with sulphate and excreted in the urine. This is based on studies on the structurally related substances cyclopentanone, cyclohexanone and cycloheptanone, which were given by gavage to rabbits (1.7–2.3 mmol/kg) and rats (1.8–2.5 mmol/kg), and 1–3 % of the dose was found in the urine as sulphate conjugates (James and Waring, 1971).

D.3 Summary and conclusions

The 11 esters [FL-nos: 09.154, 09.355, 09.520, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929, 09.935 and 09.949] included in this FGE are expected to be hydrolysed to the corresponding carboxylic acids and alcohols, based on the evaluation of supporting substances (Heymann, 1980; Anders, 1989; White et al., 1990; Emberger, 1994a, b). The resulting carboxylic acids are either metabolised through common physiological pathways, such as beta-oxidation and the citric acid cycle, or excreted in conjugation with glucuronide (Keefer et al., 1987; Vree et al., 1994).

The one hemiketal ester [FL-no: 06.136] is expected to be hydrolysed to the corresponding cyclic ketone, p-menthan-3-one [FL-no: 07.059], and lactic acid [FL-no: 08.004].

One of the main pathways for the candidate alcohols and the ketones (after reduction) [FL-nos: 02.070, 02.075, 02.135, 02.167, 07.059, 07.109, 07.202, 07.203, 07.219 and 07.255] is conjugation with glucuronic acid followed by excretion. Menthol, carveol and dihydrocarveol, hydrolysis products of [FL-nos: 06.136, 09.154, 09.355, 09.618, 09.619, 09.621, 09.843, 09.870, 09.929 and 09.935], are also metabolised via this pathway. Neither menthol nor carveol or dihydrocarveol is anticipated to be oxidised to the corresponding ketone.

Additional pathways involved in the metabolism of the candidate substances are reduction of ketone groups, oxidation of alkyl groups of alkyl-substituted alicyclic ketones followed by conjugation with glucuronic acid and/or sulphates resulting in excretion.

Thus, it may be anticipated that these 22 substances will be metabolised to innocuous products.

Appendix E. Toxicity data

Table 14: Acute toxicity

Chemical Name [FL-no] [(a)]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
Menthol [02.015]	Mouse	M	Gavage	2 652	Food and Drug Research Laboratories, Inc. (1975)	
	Mouse	M	Gavage	4 384	Food and Drug Research Laboratories, Inc. (1975)	
	Mouse	NR	Gavage	3 100	Wokes (1932)	
	Rat	M, F	Gavage	3 180	Jenner et al. (1964)	
	Rat	M	Gavage	940	Food and Drug Research Laboratories, Inc. (1975)	
trans-Menthone [07.176]	Rat	M, F	Oral	1 600–1 950	(Levenstein (1973a), Igimi and Ide (1974)	Test material = racemic menthone
Menthyl acetate [09.016]	Rat	M, F	Gavage	> 7 000	Levenstein (1973b)	Test material = racemic menthyl acetate
	Rat	M, F	Oral	> 5 000	Shelanski (1972)	Test material = *l*-menthyl acetate
Dihydrocarveol [02.061]	Rat	NR	Oral	> 5 000	Moreno (1977)	
Dihydrocarvyl acetate [09.216]	Rat	NR	Oral	> 5 000	Moreno (1980)	
neo-Dihydrocarvyl acetate [09.355]	Rat	NR	Oral	> 5 000	Moreno (1980)	
Cyclopentanol [02.135]	Rat	NR	Gavage	< 625	Myers et al. (1980)	
3,5,5-Trimethylcyclohexanol [02.209]	Rat	M, F	Oral	3 250	Smyth and Carpenter (1948)	
Cyclohexanone [07.148]	Rat	M, F	Oral	1 705	Kohli et al. (1967)	
	Rat	M, F	Oral	1 840	Deichmann and LeBlanc (1943)	
	Rat	M, F	Oral	1 620	Smyth et al. (1969)	
	Rat	M, F	Oral	1 800	Gupta et al. (1979)	
	Mouse	M, F	Oral	2 070	Gupta et al. (1979)	
	Rabbit	M	Gavage	1 600	Treon et al. (1943)	
	Rabbit	M	IP	1 540	Gupta et al. (1979)	
	Guinea pig	M	IP	930	Price (1951)	

Table 14: Acute toxicity

Chemical Name [FL-no] [(a)]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
Cyclohexanol [02.070]	Rat	M	Gavage	1 750	Miller and Sherman (1965)	
	Rat	NR	Oral	1 550	Birch (1978)	
	Rat	NR	Oral	2 060 [(b)]	Smyth et al. (1946)	
	Rat	NR	Oral	2 060	Bär and Griepentrog (1967)	
	Rat	M, F	Oral	1 120	Birch et al. (1981)	
	Rabbit	NR	Gavage	2 200–2 600 [(c)]	Treon et al. (1943)	
3-Methylcyclopentadecan-1-one [07.111]	Dog	M, F		> 2 000	You et al. (1997)	
	Rat	M, F		> 5 000	Oh et al. (1997)	
6-Isopropyl-3,9-dimethyl-1,4-dioxyspiro[4.5]decan-2-one [06.136]	Rat	NR	Oral	> 2 000	Flavour Industry (2006a)	
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Rat	NR	Oral	> 2 000	Flavour Industry (2006a)	
Carveol [02.062]	Rat	NR	Oral	3 000	Keating (1972)	
Carvyl acetate [09.215]	Rat	NR	Oral	> 5 000	Levenstein (1976)	

F, female; M, male; NR, not reported.
(a): Supporting substances are listed in brackets.
(b): Administered as 10 % solution in Tergitol 7.
(c): Minimum lethal dose.

Table 15: Subacute/subchronic/chronic/carcinogenicity studies

Chemical Name [FL-no][(a)]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg per day)	Reference	Comments
(Menthol [02.015])	Mouse; M, F 2/50	Diet	2 000, 4 000 ppm	103 weeks	600 [(b)]	National Cancer Institute (1979)	Good quality
	Mouse; F 2/30	Intraperitoneal injection (IP)	500 and 2 000 mg/kg three times week	24 weeks	A NOAEL was not determined	Stoner et al. (1973)	Good quality
	Rat; M, F 3/20	Gavage	0, 200, 400 and 800 mg/kg bw day	28 days	< 200 [(c)]	Thorup et al. (1983)	Relative good quality
	Rat; M, F 2/80	Diet	100 and 200 mg/kg bw	5.5 weeks	200 [(b)]	Herken (1961)	Limited information
	Rat; M, F 2/50	Diet	3 750 and 7 500 ppm	103 weeks	375 [(b)]	National Cancer Institute (1979)	Good quality
trans-Menthone [07.176]	Rat; M, F 3/20	Gavage	200, 400 and 800 mg/kg bw day	28 days	400	Madsen et al. (1986)	Good quality
	Mouse; F 2/30	IP	1 900 and 4 750 mg/kg three times week	24 weeks	A NOAEL was not determined [(c)]	Stoner et al. (1973)	Good quality
Cyclohexanone [07.148]	Mouse; M, F 7/20	Drinking water	400–47 000 ppm	13 weeks	M: approx. 3 300, F: approx. 6 500	Lijinsky and Kovatch (1986)	Good quality
	Rat; M, F 7/10	Drinking water	190–6 500 ppm	25 weeks	Approx. 330	Lijinsky and Kovatch (1986)	Good quality
	Mouse; M, F 3/84–104	Drinking water	6 500, 13 000 and (F) 25 000 ppm	2 years	Approx. 1 600	Lijinsky and Kovatch (1986)	Good quality
	Rat; M, F 2/104	Drinking water	3 300 and 6 500 ppm	2 years	Approx. 330	Lijinsky and Kovatch (1986)	Good quality
	Rat 1/7	IP	200 mg/kg bw (twice a day) five days/week	13 weeks	A NOAEL was not determined [(b)]	Perbellini et al. (1981)	Only neurotoxicity was checked. Limited experimental design

Table 15: Subacute/subchronic/chronic/carcinogenicity studies

Chemical Name [FL-no][(a)]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg per day)	Reference	Comments
Cyclohexanol [02.070]	Rats 1/7	IP	200 mg/kg bw (twice a day) five days/week	3 weeks (twice a day) plus 3 weeks (once a day)	A NOAEL was not determined [(b)]	Perbellini et al. (1981)	Limited experimental design
	Rat; M 1/6	Gavage	455 mg/kg day	7 days	455 [(b)]	Lake et al. (1982)	Limited quality
	Rat; M 1/NR	Drinking water	10 ppm	30 days	1 [(b)]	Messiha and Lox (1985)	Limited quality
2-sec-Butylcyclohexanone [07.095]	Rat 3/NR	Diet		91 days	370	Hummler (1969)	Study not available
3-Methylcyclopentadecan-1-one [07.111]	Rat, M, F 3/20	Gavage		30 days	1 000 [(d)]	Oh et al. (1997)	
	Dog, M, F 3/6	Gavage		28 days	20 [(d)]	You et al. (1997)	
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Rat M, F 10/10	Diet	0, 10, 50 or 100 mg/kg bw day	90 days	100	Kelly and Bolte (2000)	

IP, intraperitoneal injection; F, female; M, male; NR, not reported

(a): Supporting substances are listed in brackets.

(b): The study was performed at a single dose level or multiple dose levels that produced no adverse effects.

(c): The test substance was administered three times per week for eight weeks; animals were observed for an additional 16 weeks.

(d): Study was performed with either a single dose or multiple doses that produced no adverse effect. The value is therefore not a true no observed effect level (NOEL), but is the highest dose tested that produced no adverse effects. The actual NOEL may be higher.

Table 16: Developmental and reproductive toxicity studies

Chemical Name [FL-no][(a)]	Study type Duration	Species/Sex No/group	Route	Dose levels (mg/kg per day)	NOAEL (mg/kg per day) Including information on possible maternal toxicity	Reference
(Menthol [02.015])	Teratology Gestation days 6–15	Mouse; F 22	Gavage	0, 1.85, 8.59, 39.9, 185	185 [(b)]	Food and Drug Research Laboratories, Inc. (1973)
	Teratology Gestation days 6–15	Rat; F 22–23	Gavage	0, 2.18, 10.15, 47.05, 218	218 [(b)]	Food and Drug Research Laboratories, Inc. (1973)
	Teratology Gestation days 6–15	Hamster; F 20–22	Gavage	0, 4.05, 21.15, 98.2, 405	405 [(b)]	Food and Drug Research Laboratories, Inc. (1973)
	Teratology Gestation days 6–18	Rabbit; F 9–11	Gavage	0, 4.25, 19.75, 91.7, 425	425 [(b)]	Food and Drug Research Laboratories, Inc. (1973)
Cyclohexanol [02.070]	Reproductive NR [(c)]	Mouse; M, F NR	Diet	ca. 1 500 (1 %)	< 1 500 (< 1 %)	Gondry (1972)

F, female; M, male; NR, not reported.
(a): Supporting substances are listed in brackets.
(b): The study was performed at a single dose level or multiple dose levels that produced no adverse effects.
(c): Animals were exposed during gestation, lactation and weaning over multiple generations. Total length of exposure not reported.

Table 17: Genotoxicity (*in vitro*)

Chemical name [FL-no] [(a)]	Test system	Test object	Concentration	Result	Reference	Comments
(Menthol [02.015])	Ames test	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	0, and 6 concentrations up to 5 000 µg/plate	Negative [(b)]	Ishidate et al. (1984)	*d,l*-Menthol was used. The study is considered valid
	Ames test (pre-incubation method)	*S. typhimurium* TA97, TA98, TA100, TA1535	3–666 µg/plate	Negative [(b)]	Zeiger et al. (1988)	*d,l*-Menthol was used. The study is considered valid
	Ames test	*S. typhimurium* TA98, TA100, TA2637	0, 5–500 µg/plate	Negative [(b)]	Nohmi et al. (1985)	*d,l*-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid
	Ames test	*S. typhimurium* TA98, TA100, TA2637	0, 20–500 µg/plate	Negative [(b)]	Nohmi et al. (1985)	*l*-Menthol was tested. The highest concentrations were cytotoxic. The study is considered valid
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 6.4, 32, 160 and 800 µg/plate	Negative [(b)]	Andersen and Jensen (1984)	No indication of which enantiomer was used. In the absence of metabolic activation, the highest concentration was cytotoxic. The study is considered valid
	Ames test	*E. coli* WP2 *uvrA* (Trp-)	100–800 µg/plate	Negative	Yoo (1986)	*l*-Menthol was used. The article is not in English. The validity of the study cannot be evaluated. It is unclear whether metabolic activation or a control group was used
	Ames test	*S. typhimurium* TA97A, TA98, TA100, TA102	0, 5–800 µg/plate	Negative [(b)]	Gomes-Carneiro et al. (1998)	(-)-Menthol was used. The range of concentrations tested varied between the different strains. Cytotoxicity was observed with the highest concentrations tested with TA97A and, in the presence of metabolic activation, the highest concentration tested with TA102. The study is considered valid
	Rec assay	*B. subtilis* H17, M45	Up to 10 000 µg/disk	Positive	Yoo (1986)	*l*-Menthol was used. Inhibition zone for *rec*– and *rec*+ was 42 and 23 mm, respectively. The article is not in English. It is not clear from the study whether metabolic activation or a control group was used. The validity of this study cannot be assessed. The method (*rec*-assay) has poor predictive value
	Rec assay	*B. subtilis* H17, M45	20 µg/disk	Negative	Oda et al.	*l*-Menthol was used. The article is not in English.

Table 17: Genotoxicity (*in vitro*)

Chemical name [FL-no] [a]	Test system	Test object	Concentration	Result	Reference	Comments
					(1979)	Only one concentration level is mentioned in a table. No data on metabolic activation or control group. The validity of this study cannot be evaluated. The method (*rec*-assay) has poor predictive value
	Alkaline elution assay	Rat hepatocytes	0, 0.1–1.3 mM (203.2 µg/ml [c])	Negative	Storer et al. (1996)	The experiment employed d-Menthol. An increase in DNA breaks was observed only at concentrations associated with cytotoxicity. The authors concluded that this was a false-positive result. The study is considered valid
	Sister chromatid exchange	Chinese hamster ovary cells	5–50 and 0, 2–25 µg/ml [d] 0, 16–167 µg/ml [e]	Negative [b]	Ivett et al. (1989)	*d,l*-Menthol was used. The compound was tested up to toxic or near-toxic concentration levels. The study is considered valid
	Sister chromatid exchange	Human lymphocytes	0, 0.1, 1, 10 mM (1 563 µg/ml [c])	Negative [b]	Murthy et al. (1991)	The study is considered valid
	Cytogenetic assay	Human embryonic lung cells	0, 0.1, 1, 10 µg/ml	Negative	Food and Drug Research Laboratories, Inc. (1975)	The report does not mention exogenous metabolic activation. The study is considered valid
	Chromosome aberration	Chinese hamster fibroblasts	0 and three concentrations up to 200 µg/ml	Negative [d]	Ishidate et al. (1984)	The maximum concentration (cytotoxic) was selected by a preliminary test. The study is considered valid
	Chromosome aberration	Chinese hamster ovary cells	0, 50–250 µg/ml	Negative [b]	Ivett et al. (1989)	*d,l*-Menthol was used. The compound was tested up to toxic or near-toxic concentration levels. The study is considered valid
	Chromosome aberration	Human lymphocytes	0, 0.1, 1, 10 mM (1 563 µg/ml [c])	Negative [b]	Murthy et al. (1991)	The study is considered valid
	Gene mutation assay	Mouse lymphoma L5178Y TK+/– cells	0, 12.5–200 µg/ml	Negative [b]	Myhr and Caspary (1991)	*d,l*-Menthol was used. The maximum concentration was selected by a preliminary test The study is considered valid

Table 17: Genotoxicity (*in vitro*)

Chemical name [FL-no] [(a)]	Test system	Test object	Concentration	Result	Reference	Comments
trans-Menthone [07.176]	Ames test	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	0, 6.4–800 µg/plate	Positive [(b)]	Andersen and Jensen (1984)	Concentrations were selected based on preliminary experiments. In the absence of metabolic activation, menthone was mutagenic only to strain TA1537 at 6.4 and 32 µg/ml (slightly less than twofold increase in mutation frequency), but not at higher (toxic) concentrations. In addition, in the absence of metabolic activation, there was a concentration-dependent increase in number of TA97 strain revertants (up to fourfold increase at 600 µg/l). It was stated that metabolic activation did not enhance the mutagenicity of menthone. The study is considered valid
Cyclopentanol [02.135]	Modified Ames test	*S. typhimurium* G46, TA98, TA100, TA1535, C3076, TA1537, D3052, TA1538 *E. coli* WP2, WP2 *uvrA*–	0, 0.1–1 000 µg/ml	Negative [(b)]	McMahon et al. (1979)	The study was performed with agar plates containing the following concentration gradients: 0.1–1, 1–10, 10–100 and 100–1 000 µg/ml. The study is considered valid, although tabulated data on cyclopentanol were not presented
(Cyclohexanone [07.148])	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33–3 333 µg/plate	Negative [(b)]	NTP (2007)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 33–10 000 µg/plate	Negative [(b)]	Haworth et al. (1983)	The highest level tested was the highest of either 10 000 µg/plate, limit of solubility or maximal non-toxic concentration. The test was run twice. Both rat and hamster liver S9 were used. The test is considered valid
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0, 3 µmol/plate	Negative [(b)]	Florin et al. (1980)	A preliminary assay was performed with the four strains using only one concentration level (3 µmol/plate). This assay gave uncertain results. In addition, strains TA98 and TA100 were exposed to 0.03–30 µmol/plate. The validity of the study cannot be evaluated

Table 17: Genotoxicity (*in vitro*)

Chemical name [FL-no] [(a)]	Test system	Test object	Concentration	Result	Reference	Comments
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	NR	Positive	Massoud et al. (1980)	Only an abstract is available. No reporting with respect to metabolic activation. The substance was also tested with *B. subtilis*. With this species, toxicity was found, as well as a positive response. The validity of the study cannot be evaluated because of the lack of experimental information
	Cytogenetic assay	Human leucocytes	0.1–10 mM	Inconclusive [(d)]	Collin (1971)	The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose–response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate
	Chromosomal aberration	Human lymphocytes	0, 0.005–0.1 µg/ml	Positive	Dyshlovoi et al. (1981)	Article is not in English. Only an abstract is available in English. The validity of the study cannot be evaluated
	Gene mutation (HPRT)	Chinese hamster ovary cells	0, 7.5 µg/ml	Negative [(b)]	Aaron et al. (1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated
	Chromosomal aberration	Chinese hamster ovary cells	0, 7.5 µg/ml	Negative [(b)]	Aaron et al. (1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated
	Sister chromatic exchange	Chinese hamster ovary cells	0, 7.5 µg/ml	Positive [(d)] Negative [(e)]	Aaron et al. (1985)	Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated
	Mutation	Mouse lymphoma *L5178Y* TK+/– cells	312.5–5 000 µg/ml	Negative	NTP (2007)	
Cyclohexanol [02.070]	Ames test	*S. typhimurium* TA98, TA1535, TA1537, TA1538	500–10 000 µg/plate [(d)] 500–15 000 µg/plate [(e)]	Negative [(b)]	Barsky (1976)	The highest concentrations showed cytotoxicity. The study is considered valid

Table 17: Genotoxicity (*in vitro*)

Chemical name [FL-no] [(a)]	Test system	Test object	Concentration	Result	Reference	Comments
	Ames test	S. typhimurium TA98, TA100, TA1535, TA1537	0, 10–3 333 µg/plate	Negative [(b)]	Haworth et al. (1983)	The highest level tested was the highest of either 10 000 µg/plate, limit of solubility or maximal non-toxic concentration. Both rat and hamster liver S9 were used. The test was run twice. The study is considered valid
	Chromosomal aberration	Human leucocytes	0.1–10 mM	Inconclusive [(d)]	Collin (1971)	The study report contains little experimental detail. Gaps, but no increase in breaks, were observed without any dose–response relationship. There was no information with respect to cytotoxicity or presence of a control group. Only a statement on observations from 12 cells per concentration was given, but the total number of cells studied was not specified. The study is inadequate
(Cyclohexyl acetate [09.027])	DNA damage	*B. subtilis* H17(*rec*$^+$), M45 (*rec*$^-$)	19 mg/disc	Negative [(b)]	Yoo (1986)	
(Cyclohexyl butyrate [09.230])	DNA damage	*B. subtilis* H17(*rec*$^+$), M45 (*rec*$^-$)	19 mg/plate	Negative [(b)]	Oda et al. (1979)	
(Cycopentanone [07.149])	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	2.5–2 500 mg/plate	Negative [(b)]	Florin et al. (1980)	
(2,2,6-Trimethyl cyclo-hexanone [07.045])	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	4.2–3 600 mg/plate	Negative [(b)]	Florin et al. (1980)	
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Reverse mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	5 mg/plate	Negative [(b)]	Thompson (2000)	Valid study in compliance with the OECD Guideline 471
	Reverse mutation	*E. coli* WP2 *uvrA*	5 mg/plate	Negative [(b)]	Wagner and Klug (2000)	Valid study in compliance with the OECD Guideline 471
	Forward mutation Test	Mouse lymphoma cells *L5178y*	200 or 300 µg/l 300 µg/l	Positive [(d)] Positive [(e)]	Ross and Harris (1979)	Pre-GLP study—not possible to assess the reliability of these studies.

Table 17: Genotoxicity (*in vitro*)

Chemical name [FL-no] [(a)]	Test system	Test object	Concentration	Result	Reference	Comments
	Forward mutation Test	Mouse lymphoma cells *L5178y*	100–325 µg/l	Negative [(b)]	Cifone (2001)	Valid study and in compliance with OECD Guideline 476
(Carveol [02.062])	Ames test (pre-incubation)	*S. typhimurium* TA98, TA100, TA1535, TA1537	560 µg/plate	Negative [(b)]	Mortelmans et al. (1986)	
(Carvyl acetate [09.215])	Ames test (pre-incubation)	*S. typhimurium* TA98, TA100, TA1535, TA1537	333 µg/plate	Negative [(b)]	Mortelmans et al. (1986)	
(*L*-Menthyl (*R*,*S*)-3-hydroxybutyrate)	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	78, 156, 312, 625, 1250, 2 500 or 10 000 µg/plate	Negative [(b)(f)]	Morimoto (2005)	The JECFA evaluated the racemate of *L*-menthyl (*R*,*S*)-3-hydroxybutyrate
	Reverse mutation	*E. coli* WP2 *uvrA*	78, 156, 312, 625, 1 250, 2 500 or 10 000 µg/plate	Negative [(b)(f)]	Morimoto (2005)	

HPRT, hypoxanthine-guanine phosphoribosyltransferase gene; NA, not applicable; NR, not reported.
(a): Supporting substances are listed in brackets.
(b): With and without S9 metabolic activation.
(c): Calculated based on molecular weight of menthol = 156.3 g/mol.
(d): Without S9 activation.
(e): With S9 activation.
(f): Modified pre-incubation method.
(g): Marked differential toxicity was seen at dose levels above 25 µmol/plate. No observations were noted at lower dose levels.

Table 18: Genotoxicity (*in vivo*)

Chemical Name [FL-no][(a)]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(Menthol [02.015])	Host mediated mutation assay	*S. typhimurium* TA1530 and G46; *S. cerevisiae* D3 inoculated in mice (7-9 animals/group)	Gavage	0, 1.45 - 5000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Equivocal	Food and Drug Research Laboratories, Inc., 1975	Negative results, with the exception of the combination *S. typhimurium* TA1530 – 5000 mg/kg bw and *S. cerevisiae* D3 – 1150 mg/kg bw per day. This study is considered valid, but the equivocal result might have low relevance since the effect was observed at only very high (lethal) dose levels.
	In vivo cytogenetic assay	Male rat bone marrow cells	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Negative	Food and Drug Research Laboratories, Inc., 1975)	Oral DL_{50} was determined as 940 mg/kg bw. The study is considered valid but the negative result is of limited relevance, since no effect on mitotic index was observed. However, testing at higher dose levels may not have been possible, because of lethality.
	In vivo micronucleus assay	B6C3F1 male mouse bone marrow cells	Intra peritonal	0, 250 - 1000 mg/kg bw per day, for 3 days	Negative	Shelby et al., 1993	*d,l*-Menthol was used. The study is considered valid, but the negative result is of limited relevance, since no toxicity to the bone marrow was observed. However, testing at higher dose levels was not possible, because the highest dose caused 50 % lethality.
	In vivo dominant lethal assay	Male rat fertility, spermatozoa	Gavage	0, 1.45 - 3000 mg/kg bw (single dose) 0, 1150 mg/kg bw per day (repeated doses)	Negative	Food and Drug Research Laboratories, Inc., 1975	This study is considered valid.

Table 18: Genotoxicity (*in vivo*)

Chemical Name [FL-no][(a)]	Test System	Test Object	Route	Dose	Result	Reference	Comments
(trans-Menthone [07.176])	*In vivo* SMART assay	*D. melanogaster* – flr3 × mwh cross	Whole body	0, 1.3 µl/disk	Positive	Franzios et al., 1997	Somatic Mutation and Recombination Test. Only one dose level (1.29 µl/disk; slightly higher than the LD_{50}) was tested. A two-fold increase in mutation frequency as compared with control was observed. Menthone was not recombinogenic. The validity of this study is unclear.
(Cyclohexanone [07.148])	*In vivo* sex-linked recessive lethal mutation	*D. melanogaster*	NR 3 days exposure	0, 1 µl/ml	Negative	Goncharova, 1970	Article in Russian. Only an abstract available in English. The validity of this study cannot be assessed.
Cyclohexanol [02.070]	*In vivo* sex-linked recessive lethal mutation	*D. melanogaster*	NR 3 days exposure	0, 1 µl/ml	Negative	Goncharova, 1970	The validity of the study cannot be evaluated.
	In vivo micronucleus test	NMRI mouse bone marrow	Oral	500 - 1500 mg/kg bw	Negative	Gelbke, 1991	The study is considered valid. The negative result of this study is of limited relevance, since no bone marrow toxicity could be detected. Testing at higher dose levels might not have been possible because of observed general toxicity at the highest dose.
Methyl 3-oxo-2-pentyl-1-cyclopentylacetate [09.520]	Micronucleus test	ICR mice	Intraperitonal	280, 560 or 1120 mg/kg bw	Negative	Gudi and Krsmanovic, 1998	Valid study in compliance with the OECD Guideline 474.
	Unscheduled DNA Synthesis	Rat hepatocytes	Intraperitonal	333.3 or 1000 mg/kg bw	Negative	Durward, 2001	Valid study in compliance with the OECD Guideline 486.

NR: Not reported.
(a): Supporting substances are listed in brackets.